Hematology and Serum Chemistry of Free Trapped Five-Stripes Palm Squirrel *(Funambulus pennanti)*

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ABSTRACT

Free-roaming five-striped squirrels (*Funambulus pennanti*), an invading species in Israel, were trapped in the Ramon crater, Negev desert, anesthetized and blood samples were collected for comprehensive hematology and serum chemistry analyses, which are described herein, and compared with data obtained for other squirrel species. Suggested reference intervals (RIs) were calculated. Polychromasia and Howell-Jolly bodies (HJB) were common findings, while mild metarubricytosis was present in 6/45 squirrels. In 30/53 animals (57%) intra-erythrocyte basophilic polygonal granular inclusions resembling HJBs in size and color were noted. The total leukocytes count was positively and significantly moderately to strongly correlated with the absolute neutrophil, monocyte and lymphocyte counts. The neutrophil and monocyte morphologies resembled that of their corresponding canine cells. Eosinophils were uncommon, and resembled porcine eosinophils, while basophils showed intense metachromasia and were rare. The ranges of the results of serum amylase, alkaline phosphatase (ALP), creatine kinase (CK) activities and of serum creatinine, phosphorus and urea concentrations were very wide. Serum total bilirubin (tBr) was >7 mg/dL in 41/41 squirrels. The proposed RI for β-hydroxybutyric acid is relatively higher compared to other species (0.5-3.7 mmol/L). There were significant weak to moderate positive correlations between several serum chemistry analytes, but not between serum creatinine and urea concentrations. Muscle alanine aminotransferase content in the five-striped palm squirrel was low and most of its activity was likely of hepatocellular origin. gamma-glutamyl-tranferase (GGT) activities in the five-striped squirrel was low. Serum ALP activity and serum phosphorus concentrations were moderately correlated and both analytes were higher in young animals compared to adults.

Keywords: Squirrel; Hematology; Serum Chemistry; Clinical Pathology; Wildlife.

INTRODUCTION

In year 2001, during a routine tour by an Israel Nature and Parks Authority (INPA) ranger in the Ramon crater, Negev desert, south Israel, free roaming squirrels were observed. Since this species were not native, an investigation as to their origin was conducted, which revealed that the squirrels originated from a nearby farm. Squirrels were imported from India by the farm owner, with a proper license from the INPA, and were intended to be kept in captivity in a small zoo within the farm premises. However, three squirrels escaped captivity to the nearby desert environment and multiplied. The INPA defined these squirrels an invading species, endangering the fauna in the Ramon crater region, and initiated an effort to capture the free-roaming squirrels. Over the next several months over 200 squirrels were trapped by INPA rangers. Whole blood samples were obtained from three of the trapped squirrels for polymerase chain reaction DNA analysis. Part of their genome was sequenced and these sequences were compared to NCBI gene bank sequences by Basic Local Alignment Search Tool (BLSAT) search. Based

on the results, the squirrels were speciated as five-striped palm squirrels (*Funambulus pennanti*; family, *Sciuridae*; subfamily, *Callosciurinae*; tribe, *Funambulini*; genus, *Funambulus*; subgenus; *Prasadsciurus*), also known as Northern palm squirrels.

Based on more recent molecular studies, using two mitochondrial genes and one nuclear gene, all squirrel species were divided into five tribes. The five-striped squirrel is included among the Indo-Malayan tree squirrels tribe (1, 2). The natural geographic distribution of the five-striped squirrel is Southeast Asia, including India, Pakistan, Nepal, Bangladesh and Iran (3-5). Adult five-striped squirrels weigh between 135 to 200 grams with a length is 20 to 30 cm, of which approximately half consists of its tail.

The INPA initiated an investigation of their biology in order to assess their impact on the local ecology. This study describes the hematology and serum biochemistry of trapped, free-roaming five-striped palm squirrels trapped in the Ramon crater, south Israel. To the best of the authors' knowledge, this is the first study of the hematology and serum biochemistry of this species.

MATERIALS AND METHODS

Ethical statement

The five-stripe palm squirrel is considered by the INPA an invading species, endangering the local fauna in the Ramon crater region of Israel. The INPA has therefore decided to eliminate these squirrels from this region and conduct a study of their biology. The latter included a comprehensive analysis of their hematology and serum chemistry. The squirrels included in the present study were captured by INPA rangers and later anesthetized. Under anesthesia, blood samples were obtained, and the squirrels were later euthanized in a humane manner, as required under national and international standards. The whole procedure of blood sampling was done with minimizing stress and pain. These procedures were performed by the Chief Veterinarian of the INPA.

Animals and sampling

Squirrels were captured in feeding traps, during September 2001, and were anesthetized within 12 hours of trapping. The entire trap was covered with a sealed plastic bag, and squirrels were anesthetized using 5% isoflurane (Forane, Baxter, Mississauga, Ontario) in 100% oxygen (5 L/min), after which ketamine (Fort Dodge, Iowa, USA) was administered intramuscularly (20 mg/animal). Whole blood samples (2 to 3 mL per animal) were collected via cardiac puncture, into potassium-EDTA tubes and plain tubes containing no anticoagulant, with gel separators, for hematology and serum chemistry analyses, respectively.

Laboratory methods

Complete blood counts were performed using an impedance hematology analyzer (Abacus, Diatron, Wien, Austria), set for rat blood, within 30 minutes from collection. Blood smears were prepared within 30 minutes from collection, were air-dried, and stained with a modified Wright's staining solution (Modified Wright's stain, Bayer Hematek 2000 Slide Stainer, Bayer Diagnostics, Elkhart, IN, USA), and used for manual 100-cell differential leukocyte counts, blood cell morphological assessment and platelet count estimation. The level of polychromasia was assessed semi-quantitatively (mild, 3-5 cells/field; moderate, 6-9 cells/field; marked, >9 cells/field). When platelet clumping was observed or when the estimated platelet count differed >15% from the automated platelet count, the latter was omitted from the statistical analysis. The packed cell volume (PCV) was measured after centrifugation of heparinized capillary tubes. Total plasma protein (TPP) was measured by refractometery (Atago, Tokyo, Japan).

Whole blood samples for serum chemistry were allowed to clot, centrifuged within 60 minutes from collection, and harvested sera were analyzed immediately at 37°C (Cobas-Mira, Roche, Mannheim, Germany). Serum electrolytes were measured using an ion-selective electrolyte analyzer (OmniC, Roche, Mannheim, Germany). Hemolyzed samples were excluded.

Statistical methods

The distribution pattern of continuous variables was determined by the Kolmogorov-Smirnov test. In most cases, the proposed reference interval (RI) of analytes was calculated as the 2.5 to 97.5 inter-percentile range. For some analytes, which showed a very wide range of results, with some outliers, the proposed RI was calculated after excluding these outliers. The association between two continuous variables was examined using Pearson's or Spearman's correlation tests, for normally and non-normally distributed variables, respectively. The association between categorical and continuous variables was examined using the Kruskal-Wallis test. All tests were 2-tailed, and a $P \leq 0.05$ was considered statistically

significant. Analyses were made using a statistical software package (SPSS 17.0, SPSS, Chicago, IL, USA).

RESULTS

The study included 45 squirrels (23 males and 22 females), deemed healthy based on physical examinations (i.e. vital signs under anesthesia and their body condition), performed under general anesthesia.

Hematology

The CBC and differential leukocyte results of all squirrels, (males and females combined) are provided in Table 1, and compared to the results of other squirrel species in Table 2. Polychromasia (Figure 1 D, F) was noted in 23/45 squirrels (51%), and was mild in 16 animals (36%) and moderate in 7 (16%). In the remaining 22 squirrels, polychromasia ranged between 1 to 2 cells per X1000 field. Metarubricytes (Figure 2A) were noted in 6/45 squirrels (13%), with no evidence of anemia, based on the hematocrit (1 to 2 and 4 metarubricytes/100 leukocytes in five and one squirrels, respectively). Polychromasia was mild in four of these six squirrels, moderate in one and absent in one. Howell-Jolly bodies (HJBs) were noted in 24/45 squirrels (53%). In 30/53 animals (57%) basophilic (blue to purple) polygonal granular inclusions resembling HJBs in size and color were noted in the red blood cells (RBCs), often surrounded by a pale halo (Figure 2B). Their color resembled that of HJBs, however, they had a crystallized shape. Poikilocytosis and schistocytosis were not observed.

Platelets appeared considerably smaller than RBCs, and mostly showed a distinct central granulomer, but occasionally, uniform granularity was observed in the whole platelet

Table 1: Hematology results of apparently healthy, male and female, wild five-striped squirrels (*Funambulus pennanti*) trapped in the Ramon crater, Negev, Israel

Analyte (units)	n[1]	Median (range)	IQR[2]	Mean±SD[3]	Proposed RI[4]
White Blood Cell ($x10^9$/L)*	44	4.03 (1.21-8.44)	2.90-5.36	4.17±1.72	1.21-8.44
Neutrophils (%)*	43	70.0 (47.0-90.0)	62.0-77.0	70.6±11.3	48.04-93.16
Neutrophil bands (%)†	43	1.0 (0.0-13.0)	0.0-2.0	1.6±2.6	0-6.7[a]
Lymphocytes (%)*	43	18.0 (4.0-42.0)	11.0-24.0	19.4±9.8	0-39.06
Monocytes (%)*	43	10.0 (4.0-22.0)	7.0-14.0	10.6±4.2	2.22-18.94
Eosinophils (%)†	43	1.00 (0.0-4.0)	0.0-1.0	0.8±1	0-3
Basophils (%)†	43	0.0 (0.0-4.0)	0.0-0.0	0.2±0.8	0-1[b]
Neutrophils ($x10^9$/L)*	43	2.8 (0.6-5.7)	2.2-3.8	3±1.2	0.53-5.41
Neutrophil bands ($x10^9$/L)†	43	0.03 (0.00-1.10)	0.00-0.07	0.07±0.18	0.0-0.26[a]
Lymphocytes ($x10^9$/L)†	43	0.62 (0.08-2.87)	0.38-1.10	0.85±0.64	0.22-2.23
Monocytes ($x10^9$/L)*	43	0.41 (0.08-1.13)	0.27-0.59	0.44±0.25	0-0.94
Eosinophils ($x10^9$/L)†	43	0.03 (0.00-0.17)	0.00-0.06	0.04±0.04	0-0.14
Basophils ($x10^9$/L)†	43	0.00 (0.00-0.19)	0.00-0.00	0.01±0.03	0.00-0.04[b]
Packed cell volume (L/L)†	45	0.43 (0.30-0.51)	0.39.0-0.45	0.42±0.05	0.33-0.50
Red blood cells ($x10^{12}$/L)*	44	8.11 (5.42-10.20)	6.96-8.62	7.94±1.07	5.8-10.8
Hemoglobin (g/L)*	44	143 (99-174)	128-150	140.1±17.1	106-174
Hematocrit (L/L)*	44	0.421 (0.314-0.519)	0.375-0.437	0.413±0.046	0.320-0.506
Mean corpuscular volume (fL)*	44	52.0 (48.0-58.0)	51.0-53.0	52±2.0	47.9-56
Mean corpuscular hemoglobin(pg)*	44	17.60 (15.70-19.10)	17.00-18.20	17.60±0.87	15.9-19.3
MCHC[5](g/L)*	44	344 (294-363)	334-351	339±16	308-370
RDW[6] (%)*	44	22.1 (20.3-25.3)	21.1-22.9	22.1±1.2	19.7-24.5
Platelets ($x10^9$/L)*	41	592 (27-1343)	484-690	579±252	171-1066[c]
Mean platelet volume(fL) †	44	8.2 (7.3-17.9)	7.70-8.98	8.96±2.29	7.3-14.8

1, number of squirrels; 2, inter-quartile range; 3, standard deviation; 4, reference interval; 5, mean corpuscular hemoglobin concentration; 6, red blood cell distribution width; *, normal distribution; †, non-normal distribution; a, excluding one squirrel with a relative band neutrophil count of 13%; b, excluding two squirrels with relative basophil counts of 3% and 4%; c, based on 21 squirrels in which platelet clumps were absent upon blood smear examination and no threshold failure was noted in the automatic complete blood count.

Table 2: Hematology results of apparently healthy, male and female wild five-striped squirrels (*Funambulus pennanti*) trapped in the Ramon crater, Negev, Israel, compared to other squirrel species

	Five-striped squirrel *(Funambulus pennanti)*		Persian squirrel *(Sciurus anomalus)* (6)	American gray squirrel *(Sciurus carolinensis)* (8)	British gray squirrel *(Sciurus carolinensis)* (12)		Canadian red squirrel *(Tamiasciurus hudsonicus)* (7) Mean		Canadian gray squirrel *(Sciurus carolinensis)* (7) Mean	
Parameter (units)	**RI[1]**	**Mean/median[2] (Range)**	**Median (Range)**	**Range**	**M[3]**	**F[4]**	**M[3]**	**F[4]**	**M[3]**	**F[4]**
Erythrocytes ($x10^{12}$/L)*	5.8-10.8	7.9 (5.42-10.2)	10.1 (5.02-13.1)	4.0-10.0	NA	NA	8.44	7.8	6.9	6.8
Hemoglobin (g/L)*	106-174	140 (99-174)	129 (88-151)	83-177	NA	NA	156	153	137	135
Hematocrit (L/L)*	0.32-0.51	0.41 (0.31-0.52)	0.48 (0.44-0.69)	0.31-0.56	NA	NA	0.497	0.50	0.463	0.446
MCV[5] (fL)*	47.9-56	52 (48-58)	49.4 (38.4-58.3)	NA	NA	NA	63.3	66.3	67.4	66.2
MCH[6] (pg)*	15.9-19.3	17.6 (15.7-19.1)	14.8 (11.1-20.2)	19.3-19.9	NA	NA	18.6	19.8	19.9	20.2
MCHC[7] (g/L)*	308-370	339 (294-363)	289 (233-291)	170-368	NA	NA	293	298	296	304
RDW[8] (%)*	19.7-24.5	22.1 (20.3-25.3)	NA	NA	NA	NA	NA	NA	NA	NA
Platelets ($x10^9$/L)*	171-1066	579 (27-1343)	NA	NA	NA	NA	NA	NA	NA	NA
Mean platelet volume (fL)†	7.3-14.8	8.2 (7.3-17.9)	NA	NA	NA	NA	NA	NA	NA	NA
Leukocytes ($x10^9$/L)*	0.73-7.61	4.17 (1.21-8.44)	7.05 (4.3-10.8)	2.3	NA	NA	1.74	1.86	5.07	6
Neutrophils (%)*	48-93	70.6 (47-90)	1 (0-13)	NA	3.11±0.67	3.70±0.75	59.9	58.4	59.5	71.0
Band neutrophils (%)†	0-7	1 (0-13)	NA	NA	NA	NA	NA	NA	NA	NA
Lymphocytes (%)*	0-39	19 (4-42)	19.4 (4-42)	NA	1.93±0.40	2.02±0.42	38.9	40.7	34.6	27.6
Monocytes (%)*	2-19	11 (4-22)	10.6 (4-22)	NA	NA	NA	1.11	0.88	2.04	0.2
Eosinophils (%)†	0-3	1 (0-4)	1 (0-4)	NA	NA	NA	0.04	0.00	0.74	1.2
Basophils (%)†	0-3	0 (0-4)	0 (0-4)	NA	NA	NA	0.00	0.00	0.0	0.0
Neutrophils ($x10^9$/L) *	0.53-5.41	2.97 (0.57-5.66)	4.12 (3.06-6.26)	0.41-6.63	NA	NA	1.09	1.13	3.3	4.43
Band neutrophils ($x10^9$/L) †	0.00-0.30	0.03 (0-1.1)	NA	NA	NA	NA	NA	NA	NA	NA
Lymphocytes ($x10^9$/L) †	0.22-2.23	0.62 (0.08-2.87)	2.29 (1.10-3.82)	0.06-5.29	NA	NA	0.65	0.73	1.46	1.53
Monocytes ($x10^9$/L) *	0.00-0.94	0.44 (0.08-1.13)	0.23 (0.19-0.30)	0.00-0.04	NA	NA	0.0	0.0	0.1	0.01
Eosinophils ($x10^9$/L) †	0.00-0.14	0.03 (0-0.17)	0.09 (0.059-0.115)	NA	NA	NA	0.0	0.0	0.03	0.04
Basophils ($x10^9$/L) †	0.00-0.08	0.01 (0-0.19)	NA	0.0-0.2	NA	NA	0.0	0.0	0.00	0.00

1, proposed reference intervals based on the present study (Table 1); 2, mean or median are provided for normally and non-normally distributed results, respectively; 3, male; 4, female; 5, mean corpuscular volume; 6, mean corpuscular hemoglobin; 7, mean corpuscular hemoglobin concentration; 8, red blood cell distribution width; NA, not available; *, normal distribution; †, non-normal distribution.

area (Figure 1D and 1F). In 24/45 squirrels, small platelet clumps were observed in the blood smears, or threshold failures, as determined by the hematology analyzer were noted. Therefore, only 21 automated platelet counts were considered reliable, and used to calculate the platelet count and mean platelet volume reference intervals (RIs) (Table 1).

The total leukocytes count was positively and significantly correlated with the absolute neutrophil (r = 0.913; P <0.0001), monocyte (r = 0.703; P <0.0001) and lymphocyte (r = 0.691; P <0.0001) counts. The latter two were also positively and moderately correlated (r = 0.586; P <0.0001). The neutrophil morphology resembled that of canine neutrophils

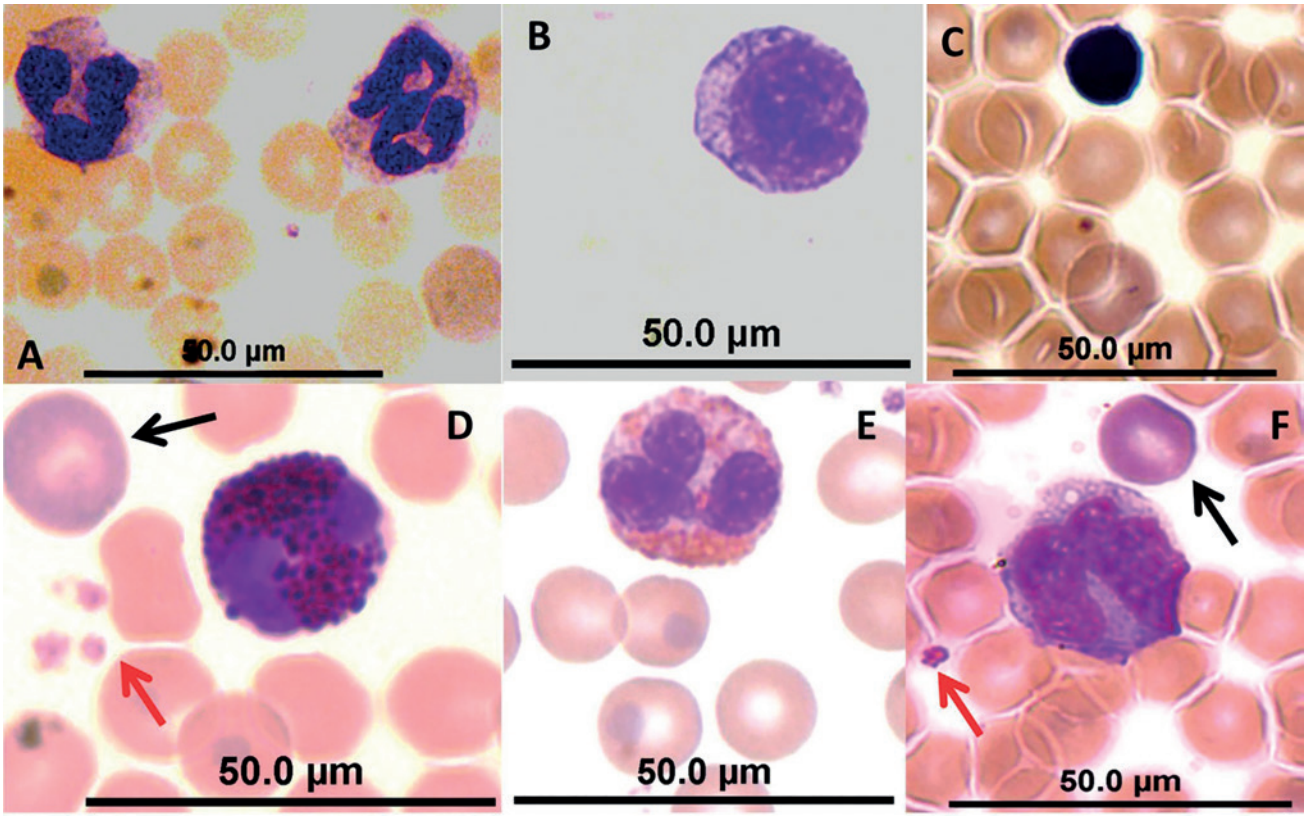

Figure 1: Leukocytes of the five-striped palm squirrel (*Funambulus pennanti*) (Modified Wright's stain). A. Two segmented neutrophils. Note the hypersementation in the neutrophil in right-hand side; B. A larger normal lymphocyte, with more abundant cytoplasm compared to most lymphocytes. These lymphocytes accounted for approximately 10% of all lymphocytes; C. Typical small lymphocyte. Such lymphocytes accounted for approximately 90% of the lymphocytes; D. Basophil. Additionally, note the polychomatophilic red blood cell (black arrow) and platelets (red arrow); E. Eosinophil. Its morphology resembles porcine eosinophils; F. Monocyte. Additionally, note the polychromatophilic red blood cell (black arrow) and a platelet (red arrow).

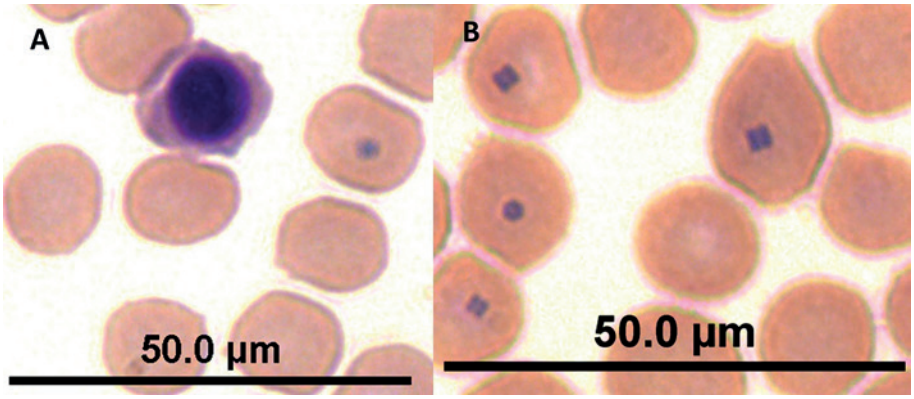

Figure 2: Red blood cells of the five-striped palm squirrel (*Funambulus pennanti*) (Modified Wright's stain).A. Metarubricyte and erythrocytes; B. Red blood cells. Note the rectangular basophilic inclusions within the red blood cells, suspected as crystalized Howell-Jolly bodies.

(Figure 1A). Generally, the number of band neutrophils was low, excluding a single squirrel, in which the relative band neutrophil count was 13%, which was considered abnormal, and was therefore omitted from the calculation of the band neutrophil RI. In most squirrels, neutrophil hypersegmentation was very common, with five to seven nuclear segments, with a minority showing eight to nine segments. Neutrophil cytoplasmic toxicity was not observed. Eosinophils were uncommon, and their morphology resembled porcine eosinophils (Figure 1E). Basophils showed intense metachromasia (Figure 1D), and were mostly rare, excluding two squirrels, in which their relative basophil counts were 3% and 4%. These were considered outliers, and were excluded from the calculation of the basophil count RI. Monocytes were the largest leukocytes (12 to 20 mm in diameter), their morphology resembling canine monocytes (Figure 1F), with round to lobulated nuclei, lacy chromatin pattern and light to deep blue cytoplasm. Most lymphocytes (90%) appeared small (6 to 9 mm in diameter), slightly larger than RBCs (4 to 6 mm in diameter), with very little or no cytoplasm (Figure 1C). Approximately 10% of all lymphocytes appeared larger by 20%, and contained more abundant cytoplasm (Figure 1B).

Table 3: Serum chemistry results of apparently healthy male and female five-striped squirrels trapped in the Ramon crater, Negev, Israel, compared to other squirrel species

		Five-striped squirrel *(Funambulus pennanti)*				Persian squirrel *(Sciurus anomalus)* (6)	American gray squirrel *(Sciurus carolinensis)* (23)
Parameter (units)	**n[1]**	**Median (range)**	**IQR[2]**	**Mean±SD[3]**	**Proposed RI[4]**	**Median (Range) (n=30)**	**Mean (Range)**
Albumin (g/dl)*	41	4.10 (1.5-9.0)	3.25-6.50	4.83±2.09	ND	NA	NA
Albumin (g/dl)*[a]	30	3.6 (1.5-5.6)	3.2-4.4	3.7±0.9	ND	NA	NA
Albumin/globulin ratio†[b]	30	2.2 (1.1-5.4)	1.8-2.5	2.4±1.0	ND	NA	NA
Alkaline phosphatase (U/L)†	41	216 (107-1020)	140-680	379±305	108-552[c]	18 (14-27)	NA
Alanine transaminase (U/L)†	41	21 (6-120)	14-34	30±25	6-94	NA	NA
Amylase (U/L)†	40	892 (34-14,450)	462-5868	3015±4131	75-8815[d]	545 (523-616)	NA
Aspartate transaminase (U/L)†	41	81 (26-492)	51-160	121±102	ND	6 (3-28)	NA
β-hydroxybutyric acid (mmol/L)*	41	2.1 (0.5-4.8)	1.5-2.6	2.1±0.8	0.5-3.7	NA	NA
Calcium, total (mg/dL)*	41	10.7 (3.0-15.5)	9.0-11.6	10.2±2.2	5.8-14.6	7.5 (7.1-8.2)	9.0 (7.2-14.5)
Chloride (mmol/L)*	27	107.1 (62.7-122.9)	94.6-116.3	103.4±16.7	ND	NA	115 (80-155)
Cholesterol (mg/dL)*	41	358(91-500)	288-420	349±95	159-539	197 (182-213)	248 (124-449)
Creatine kinase (U/L)†	41	1,134 (277-7,107)	737-2,080	1608±1,449	ND	NA	NA
Creatinine (mg/dL)†	40	0.63 (0.0-5.0)	0.20-0.89	0.91±1.09	0.3-1.50[e]	4.27 (3.92-4.62)	NA
Globulin (g/dL)*[a]	30	1.7 (0.3-2.8)	1.45-2.35	1.8±0.7	ND	NA	NA
γ-glutamyl-transferase (U/L)†	41	0.0 (0.0-18.6)	0.0-0.0	0.83±3.71	ND	NA	NA
Glucose (mg/dL) †	41	140.0 (53.0-255.0)	107.5-207.5	148.6±58.3	60-253	107 (89-125)	139 (25-255)
Potassium (mmol/L)*	27	4.6 (2.5-8.2)	4.1-5.3	4.8±1.3	ND	NA	NA
Phosphorus (mg/dL)†	41	8.2 (3.7-32.2)	6.6-23.9	12.8±9.2	3.7-11.7[f]	5.4 (3.9-6.7)	7.3 (3.1-21.7)
Sodium (mmol/L)*	27	139.0 (81-160.3)	130.0-151.0	135.3±21.8	ND	NA	NA
Total bilirubin (mg/dL)*	41	19.9 (7.0-40.8)	15.7-28.1	21.3±7.9	5.5-37.0	NA	NA
Total serum protein (gr/dL)*	41	5.90 (1.8-9.0)	5.00-6.30	5.66±1.35	2.10-5.20	7.0 (6.7-7.3)	5.5 (3.7-6.6)
Total plasma protein (g/dL)[5]†	45	6.8 (5.2-8.8)	6.0-8.0	6.9±0.95	5.24-8.0	NA	NA
Triglycerides (mg/dL)*	41	201 (51-367)	169-244	206±59	88-324	373 (364-392)	125 (20-496)
Urea (mg/dL)†	41	72.2 (21.4-300.0)	52.2-95.7	84.4±58.1	22.5-95.2[g]	33.4 (27.2-43.6)	42.8 (8.6-149.7)

1, number of squirrels; 2, inter-quartile range; 3, standard deviation; 4, reference interval; 5, determined by refractometry; NA, not available; ND, not determined (see text for details); *, normal distribution; †, non-normal distribution; a, after excluding 11 squirrels (see text for details); b, after excluding 11 squirrels (see text for details); c, proposed reference interval based on 30 squirrels, after exclusion of 11 squirrels with alkaline phosphatase activity≥680 U/L (see text for details); d, proposed reference interval based on 36 squirrels, after exclusion of 4 squirrels with amylase activity >10000 U/L (see text for details); e, proposed reference interval based on 34 squirrels, after exclusion of exclusion of squirrels with creatinine >1.2 mg/dL and urea >102 mg/dL (see text for details); f, proposed reference interval based on 30 squirrels, after exclusion of 11 outliers (see text for details); g, proposed reference interval based on 31 squirrels, after excluding 10 squirrels with urea concentration >100 mg/dL (see text for details).

Serum chemistry

The serum chemistry results of all squirrels (males and females combined) are shown in Table 3. The distribution patterns of serum alanine transaminase (ALT), alkaline phosphatase (ALP), amylase, aspartate transaminase (AST) creatine kinase (CK) and g-glutamyl-transferase (GGT) activities and serum creatinine (sCr), glucose, phosphorus and urea concentrations were not normally distributed. The ranges of the results of serum amylase, ALP and CK activities and sCr, phosphorus and urea concentrations were very wide. The distribution of serum CK activity showed no clear pattern, and it was impossible to propose a clear RI. For the proposed RI for serum amylase activity, four squirrels with activities >10000 U/L were considered outliers, and were omitted, however, even after their exclusion, the proposed amylase activity RI is markedly wide (75-8815 U/L; Table 3). Serum total bilirubin (tBr) was >7 mg/dL in virtually all 41 squirrels.

The distribution patterns of serum ALP activity and phosphorus concentration were bimodal (Figure 3), with two distinct populations, and within each, the distribution pattern was normal. Regarding ALP activity, in one population (30/41 squirrels) ALP activity ranged between 107 and 607 U/L, while for the remaining 11, the results ranged between 680 and 1020 U/L. For phosphorus concentration, one population (30/41 squirrels) showed concentrations between 3.69 to 11.59 mg/dL, while the remaining 11 squirrels showed concentrations between 23.5 to 30.9 mg/dL. Examining the individual animal data showed that the 11 squirrels with markedly higher phosphorus concentrations also had distinctly higher ALP activities. The proposed RIs for both serum ALP activity and phosphorus concentration were set based on the 30 squirrels in which the levels of both analytes were lower.

In 37/40 squirrels sCr was <1.7, and in 34/40 it was ≤1.2 mg/dL, while in the remaining eight, both sCr and urea concentrations were considerably higher compared to the rest of the squirrels (sCr range 1.22-5 mg/dL; serum urea range 102-300 mg/dL). Therefore, the latter were considered outliers, and the proposed RI for sCr was calculated excluding eight squirrels, based the remaining 34 squirrels. The proposed RI for serum urea concentration was calculated based on 31/41 squirrels, in which serum urea concentration was <100 mg/dL.

The concentration ranges of serum albumin and total protein (TP) were very wide (Table 2). In 10/41 squirrels albumin concentration was greater or equal to their TP concentration, while in another, it was 8 g/dL. These results were considered unreliable. Therefore, no RIs were proposed for serum albumin and globulin concentrations and for the albumin:globulin ratio (Table 2).

There were significant weak to moderate positive correlations between activities of AST and ALT (r = 0.725, P <0.0001), AST and CK (r = 0.54; P = 0.0002), ALT and ALP (r = 0.52; P = 0.0004) and amylase and ALP (r = 0.5; P = 0.001), as well as between amylase activity and serum phosphorus concentration (r=0.5; P=0.001), ALP activity and tBr concentration (r= 0.52; P = 0.004), ALP activity and

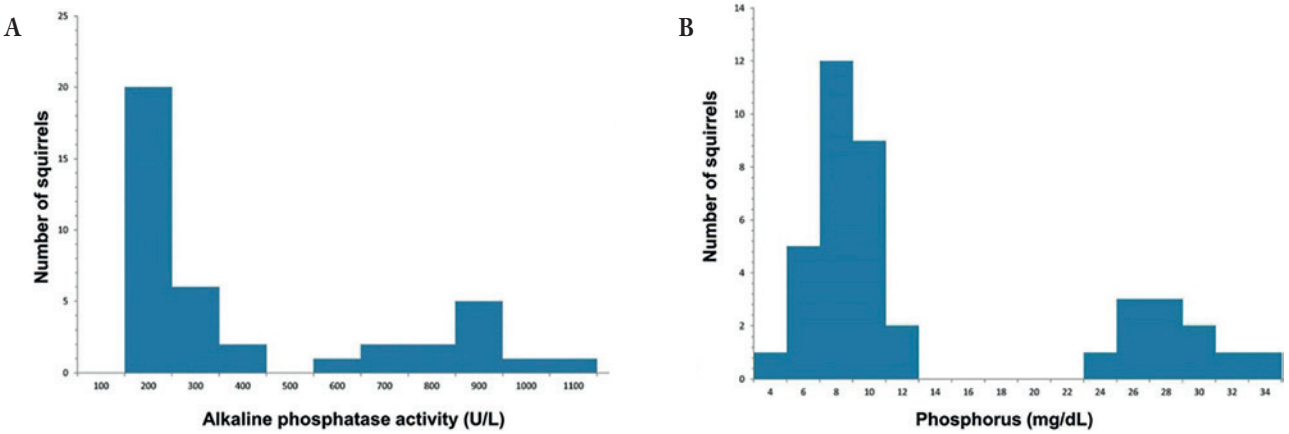

Figure 3: The distributions of serum ALP activity and phosphorus concentration in 41 five-striped squirrels (*Funambulus pennanti*), showing two distinct separate populations, in which, within both, the distribution of these two analytes was normal. We believe that the population of squirrels showing relatively lower ALP activity and phosphorus concentration were comprised of adult squirrels, while the other population, including 11 squirrels, showing higher values of these analytes (in which the ALP activity was >680 U/L) included young, growing squirrels. Therefore the proposed reference interval for ALP activity in the five-striped squirrel was based only on the 30 squirrels in which serum ALP activity was <680 U/L.

phosphorus concentration (r = 0.68; P <0.0001) and serum cholesterol and tBr concentrations (r = 0.69, P <0.0001). There were no significant correlations between serum phosphorus and sCr or serum urea concentrations and between sCr and serum urea concentrations.

DISCUSSION

This study describes, for the first time, to the best of the authors' knowledge, the hematological and serum chemistry of the five-striped squirrel, and is the most comprehensive study of laboratory analytes in squirrels in general (6-8). This study also describes for the first time the morphological characteristics of the leukocytes of this species. Nevertheless, these apparently healthy squirrels were trapped in the Negev desert, where they were considered an invading species, and not in their natural habitat, which might have influenced the laboratory results.

The results of the RBC count, the hematocrit and the hemoglobin concentrations in the five-striped squirrel in this study are similar to those reported in other squirrels (Table 2). The present mean corpuscular hemoglobin concentration and the mean corpuscular hemoglobin are slightly higher than previous results for the Persian, American gray and Canadian red and gray squirrels, while the mean corpuscular volume is similar to that of the Persian squirrel, but much lower compared to the Canadian squirrels (6-8) (Table 2).

This study, to the best of the authors' knowledge, is the first to examine the red blood cell distribution width, platelet count and mean platelet volume in squirrels in general. The high frequency of HJBs in our squirrels correlates the high frequency of polychromasia, and is similar to findings in other rodents, such as mice (9). The rectangular basophilic inclusions within RBCs may be crystalized HJBs, and their crystallization might be a species-specific morphological artifact, occurring during preparation of the blood smears. Further studies, including Prussian blue staining is warranted to determine their nature.

The median leukocyte count in the five-striped palm squirrel is considerably lower than that of 30 Persian squirrels as well as in the Canadian red squirrel, but similar to that of the American and Canadian gray squirrels (6-8) (Table 2). However, the proportions of the different leukocytes in our squirrels are similar to those reported in other squirrels, excluding the relative number of monocytes, which was presently higher (6-8) (Table 2). Relatively high mean proportions of monocytes were also reported in Arctic squirrels (*Spermophilus parryii*; 18.9% and 15.2% in males and females, respectively). The absolute differential leukocyte counts were not reported in that study (7). The variance in the leukocyte and neutrophil counts in our squirrels might be associated with several factors, including heterogeneity in age (i.e., young animals vs. adults), physiological status (i.e., pregnancy and lactation), sex, season, the diet, health status and presence of subclinical diseases and trapping procedure-associated stress and anxiety (6, 10, 11), and the impact anesthesia. For example, there were significant differences in the median leukocyte counts among different studies of gray squirrels (*Sciurus carolinensis*), conducted in the USA, Canada and the UK (6-8, 12). In the gray squirrel, the relative and absolute monocyte counts were much higher (10-fold) in males vs. females (7) (Table 2), while in other rodents, such as mice, neutrophil counts are usually higher in males vs. females (9). Stress and anxiety might have affected the present leukocyte and differential leukocyte counts, and were proposed mechanisms affecting the leukocyte counts in the Canadian gray and red squirrels (*S. carolinensis* and *Tamiasciurus hudsonicus*, respectively; Table 2) (7). In other species, including rodents, such as rats, stress and excitement dramatically affect the leukocyte count, increasing the neutrophil count, and has led to a 12% increase in the lymphocyte count (13, 14). Stress leads to glucocorticoid secretion, and in dogs it classically induces a 'stress leukogram', namely mature neutrophilia, monocytosis, eosinopenia and lymphopenia (14). A similar mechanism might have affected our squirrels, leading to relatively high proportions of neutrophils in some squirrels. This is supported by the observed high proportion of hypersegmentation (14) and the strong positive correlations between the total leukocyte count and both the neutrophil and monocyte counts. As for the physiological status, leukocyte count changes were reported during the estrus cycle in sexually-mature female rats, with leukopenia observed during estrus and neutropenia during diestrus (13). In the present study, the estrus cycle stage in the female squirrels was not determined. Additionally, although the physical examination findings of our squirrels were unremarkable, we cannot rule out presence of subclinical diseases in some animals, which might have affected the total and differential leukocyte counts. However, the proportion of band neutrophils was very low in all but one squirrel, and neutrophil cytoplasmic toxicity was virtually absent, rendering the presence of inflammation

or infection unlikely. Because the eosinophil counts were very low, parasitic diseases are also highly unlikely. Basophils were absent in the Canadian red and gray squirrels, and in ground squirrels (7), while in the American gray squirrel, the basophil proportion was similar to the present findings (6) (Table 2).

The serum chemistry analysis performed in this study is the most comprehensive one conducted in squirrels in general (6-8). Comparison of the present serum chemistry results to previous results in other squirrel species should be made cautiously, due to possible differences in the reagents, the analyzers' chamber temperature and reaction pH, all of which affect the results (15, 16).

The proposed RI for serum ALP activity in the five-striped squirrel was found to be 5 to 25-fold higher compared to the upper range of ALP activity reported in the Persian squirrel (108-552 U/L vs. 14-27 U/L, respectively). In both studies, the ALP assay used para-nitrophenyl as substrate, however, the exact reaction temperature and pH were not reported in the previous study, while in the present one, the chamber temperature was 37°C, and the pH was 10.42. Differences in the latter might have affected the results, rendering the comparison inappropriate. Nevertheless, if conditions were similar, this marked difference between studies might have resulted from a true species-related difference, or from a relatively high proportion of young growing animals in our study population, in which serum ALP activity is higher than in adults in most species (17-22), compared to the previous study. In such growing animals, serum phosphorus concentrations are also higher compared to adults, and the levels of both analytes are correlated (18). Indeed, presently, these two were significantly, positively moderately correlated ($r = 0.69$). This hypothesis is supported by examining the distributions of serum ALP activity and phosphorus concentration in our squirrels, showing two distinct separate populations, in which, within both, the distribution of these two analytes was normal (Figure 3). We believe that the population of squirrels showing relatively lower ALP activity and phosphorus concentration were comprised of adult squirrels, while the other population, including 11 squirrels, showing higher values of these analytes (in which the ALP activity was >680 U/L) included young, growing squirrels. We therefore proposed a reference interval for ALP activity based only on the 30 squirrels in which serum ALP activity was <680 U/L. Nevertheless, this RI is much higher than the serum ALP activity range in Persian squirrels (6). Similarly, for serum phosphorus concentration, the present RI is based on these same 30 squirrels, after excluding the 11 squirrels in which serum phosphorus concentration was >23 mg/dL and serum ALP activity >680 U/L. There were no significant correlations between serum phosphorus concentration and serum urea or creatinine concentrations. We therefore conclude that renal function was not a significant factor determining serum phosphorus concentration. The ALP upper reference limit (URL) as determined here is 4.5- and 1.5-fold higher than the maximal concentration reported in the Persian and gray American squirrels, respectively (6, 23), as well as in other rodents (24). Although this wide RI of serum phosphorus concentration might be a unique characteristic of the five-striped palm squirrel, it might have been influenced by the unnatural habitat of the present squirrel population.

The range of the presently measured serum albumin concentrations was wide, with some measurements being very high compared to the corresponding measured serum TP. We assume that these results represent an analytical error. Serum albumin was presently measured using the brilliant-cresyl green (BCG) assay (25, 26). It is known that under different conditions and in certain animal species, the avidity of serum proteins for BCG is variable. BCG might bind to globulins, yielding falsely high serum albumin concentration (25, 26), especially when serum albumin is relatively low, as has been reported in cows and horses (26). We therefore assume that the present serum albumin and globulin concentrations results and the calculated albumin:globulin ratio may be unreliable, even after excluding obvious outliers. The particular BCG assay used herein is probably inappropriate for albumin measurement in the five-striped palm squirrel, and therefore RIs were not proposed. Determining serum albumin and globulin concentrations in this species might be better done using serum TP concentration with concurrent serum electrophoresis, as suggested in several avian species (27).The serum TP range in this study was similar to that reported in the American gray squirrel (23), but considerably lower compared to the Persian squirrel (6) (Table 3).

The median and URL of serum glucose concentration herein were similar to its median and upper range in the American gray squirrel (23), but slightly higher compared to the Persian squirrel (6) (Table 3). Serum glucose concentration might have increased during the stress and excitement of trapping and anesthesia due to increased serum catecholamine and glucocorticoid levels (25). Such impact of stress

and excitement is particularly unavoidable when conducting studies of wildlife animal species.

This is the first study to report tBr concentration in squirrels in general. The present lower reference limit is over 6-fold higher than the tBr URL measured in other rodents (28). Since there was no evidence of hemolysis (i.e. no anemia or spherocytosis), and serum ALT activity range was unremarkable, hemolytic anemia and hepatopathy, respectively, were unlikely causes. Presence of post-hepatic diseases cannot be excluded, however, it seems unlikely to have occurred in such a high proportion of our squirrels. Serum tBr was >7 mg/dL in virtually all of the 41 squirrels. We therefore believe that this apparent hyperbilirubinemia is a species-specific physiological phenomenon. In fasting horses, hyperbilirubinemia (serum tBr up to 10 mg/dL) occurs, comprising mainly of unconjugated Br (uBr). Two responsible mechanisms have been proposed (29, 30). First, in horses, uBr is conjugated to glucose, while in other species uBr is mostly conjugated to glucoronic acid. In fasting horses, a negative energy balance occurs, and serum glucose concentrations are relatively low, thereby decreasing its conjugation to Br, and its secretion to the bile. Concurrently, serum free fatty acid (FFA) concentrations increase. These FFA are up-taken by hepatocytes from the sinusoidal blood by binding to the same receptor (ligandin) responsible for uBr uptake, and therefore, competitively inhibit hepatocyte uBr uptake. Intravenous glucose administration to fasting horses has been shown to increased serum glucose, while tBr concentration has decreased, supporting this mechanism (29). Secondly, fasting leads to a decrease in ligandin, thereby lowering uBr uptake by hepatocytes, and foals are prone to develop fasting hyperbilirubinemia because their relatively low ligandin reserve (30). During trapping, the squirrels were caged and fasted for up to 12 hours before blood sampling. Possibly, the hyperbilirubinemia observed in the five-striped palm squirrel herein is similar to that in horses. Future studies should measure both serum tBr and conjugated (direct) Br, which will allow calculating uBr (indirect Br) concentration, to examine if this hyperbilirubinemia is similar to equine fasting hyperbilirubinemia. The possibility that cholestasis-induced hyperbilirubinemia (29) occurred in our squirrels cannot entirely be rule out, because the concentrations of serum tBr and cholesterol were significantly positively correlated, while serum ALP activity range was wide. However, this seems unlikely, because serum GGT activity was markedly low in most squirrels.

The serum cholesterol concentration URL was higher than previously reported values in the five-striped squirrel (31). It was moderately correlated with serum tBr concentration, and might therefore be increased in fasting squirrels, as is proposed for serum tBr. Serum cholesterol concentration was also shown to be influenced by the reproductive cycle (31), which might have influenced the present results.

The presently proposed sCr RI was lower than its range in the Persian squirrel (6) (Table 3). Nevertheless, eight apparently healthy squirrels had higher sCr, and also showed considerably higher serum urea concentrations, and were therefore excluded from the proposed calculated sCr RI. The reasons for this azotemia are unclear. Azotemia might have been prerenal, as the squirrels were trapped and caged in the Negev desert, with very limited access to water sources, and therefore might have been dehydrated. Additionally, presence of subclinical chronic renal insufficiency cannot be excluded. Sinces Cr and serum urea concentrations did correlate with serum phosphorus concentration, this possibility cannot be resolved. Future studies should include a urinalysis, with urine specific gravity measurement, in order to assess the concentration ability of the kidneys.

Unexpectedly, there was no correlation between sCr and urea concentrations in this study. In some squirrels, serum urea concentration was considerably higher than in the remaining squirrels, and these results were considered abnormal, and were excluded from the proposed RI calculation. The present median serum urea concentration was >2-fold higher than that reported in the Persian squirrel (6) and 1.7-fold its mean concentration in the American gray squirrel (23). The question if these differences reflect a true species difference or abnormally high concentrations, possibly due to dehydration, renal insufficiency or dietary causes cannot be resolved. Measurement of both sCr and urea concentrations in the five-striped palm squirrel in its natural habitat is warranted.

The measured β-hydroxybutyric acid (BHBA) concentration in the five-striped palm squirrels (RI 0.5-3.7 mmol/L) is considerably higher than in cats (RI 0.0-0.49 mmol/L) (32), lactating cows (mean 0.41 mmol/L) (33) and the rock hyrax (*Procavia capensis*), in which these higher serum BHBA concentrations were considered characteristic of this species, probably reflecting its unique dependence on fermentation products, and the fact that volatile fatty acids account for 70% of its basal metabolic rate (34). Ketonuria was recorded in 47/160 (29%) American gray squirrels, although it was mini-

mal in most squirrels (23). This high frequency was unanticipated, and is its mechanism was unclear. Nevertheless, serum ketone concentrations were not measured in that study. The question whether the relatively high serum BHBA concentrations presently recorded in the five-striped squirrel are unique to this species, representing intensive hind-gut fermentation or have resulted from the different, unusual diet in the desert conditions, forced on the squirrels in this unnatural habitat or a negative energy balance, cannot be resolved.

As for amylase, the presently calculated RI is very wide, even after exclusion of outliers. The presently recorded amylase activity is 20-fold higher compared to that of the Persian squirrel (6), but the exact analytic method in the latter study was not specified. Differences in reagents, methods, pH and chamber temperature in the analyzer yield significant differences in the results (15, 16). As there was a weak (r = 0.5) correlation between amylase and ALP activities, and between serum phosphorus concentration (r =0.68), possibly, amylase activity was influenced by age in the five-striped squirrel, and a relatively high proportion of young squirrels in our population might have accounted for the high activities recorded in some animals. High amylase activity might also result from presence of macro-amylases, which are cleared much more slowly by glomerular filtration (35, 36). Whatever the cause of this high variability in amylase activity in the five-striped squirrel, this analyte has limited clinical usefulness in this species.

The measured GGT activity in our squirrels was lower than the detection limit, excluding two squirrels. We therefore conclude that GGT activity in the five-striped squirrel is low, and that the analytical method in insensitive for its measurement in this species.

The median AST activity herein is approximately 14-fold higher compared to the Persian squirrel (6), while that of ALT in the present study is unremarkable. Therefore, in light of the very high CK activity recorded herein, it seems that most of the recorded AST activity in our squirrels is derived from muscle (35), while the muscle ALT content in the five-striped palm squirrel is low, and is therefore most likely of hepatocellular origin. The very high CK activity recorded herein suggests that our squirrels sustained severe skeletal muscle injury, likely from combination of capture and trapping, restraint, transport, intramuscular injection and general anesthesia, as reported previously in other wildlife animals and in cats (34, 37, 38). There was a weak to moderate correlation between AST and CK activities, and both measures were not normally distributed. Therefore, it is most likely that the recorded high AST and CK activities are of skeletal muscle origin, and these do not adequately represent their true RIs in the five-striped palm squirrel.

This study has several limitations. First, the size of the squirrel population is limited, although, compared to previous studies in different squirrel species, it is larger. Second, these particular squirrels were roaming in a desert environment, which is very different to their natural habitat in the Indian subcontinent, and this likely affected the results. Third, all squirrels were genetically closely related, originating from three animals that have escaped captivity, and therefore, genetic drift can be expected, which might have affected the results. Fourth, the trapping, transport, restraint and anesthetic procedures likely affected the results of some analytes (i.e., glucose, CK, AST, tBr, cholesterol and the leukocyte count). The squirrels were physically examined under general anesthesia, which might therefore have missed certain abnormalities. Nevertheless, this issue is mostly unavoidable when investigating wildlife species. The exact age, and reproductive cycle status of the squirrels, which might have influenced the results (13, 31) were not determined. The analytical methods used were likely not appropriate for some measures (i.e., GGT activity and albumin concentration).

In conclusion, this study presents a comprehensive laboratory profile of five-striped palm squirrel, and is the largest such study in squirrel species in general. It proposes RIs for most analytes measured, although, some RIs must be applied with caution to different populations.

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