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**LRH:Hematology of western barred bandicoots**

**RRH:Bennett, Woolford, O'Hara, et al**

**Hematologic characteristics of captive western barred bandicoots (*Perameles bougainville*)  
from Western Australia**

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**Background:** The western barred bandicoot (*Perameles bougainville*) is an Australian marsupial species now considered endangered as a consequence of habitat destruction and predation. A recently discovered papillomatosis syndrome is hindering efforts to repopulate this species. Hematology reference intervals have been lacking for *P bougainville*, preventing optimal interpretation of hematology results from wart-affected and clinically normal animals.

**Objectives:** The purpose of this study was to establish hematology reference values and describe morphologic characteristics of blood cells of healthy western barred bandicoots. **Methods:** Fifty-nine whole blood samples were collected by jugular venipuncture into EDTA from 47 clinically

healthy captive western barred bandicoots at 3 locations on the Western Australian mainland. A CBC was performed using an ADVIA-120 analyzer. Data were compared on the basis of geographic location, sex, age, and lactation status, and reference intervals were calculated. Blood cell morphology was evaluated using light microscopy, and transmission and scanning electron microscopy. **Results:** Significant differences were found based on sex (RBC indices, fibrinogen), age (% polychromatophilic RBCs), and geographic location (RBC, neutrophil, and lymphocyte counts, MCHC, % polychromatophilic RBCs, fibrinogen). Combined reference intervals were calculated for hemoglobin concentration (122–165 g/L), HCT (0.36–0.49 L/L), and total WBC ( $2.9\text{--}14.9 \times 10^9/\text{L}$ ), monocyte ( $0\text{--}0.6 \times 10^9/\text{L}$ ), eosinophil ( $0\text{--}0.9 \times 10^9/\text{L}$ ), and total plasma protein (47–63 g/L) concentrations. Leukocyte, erythrocyte, and platelet morphology was similar to that of other marsupial peramelid species. Nuclei in neutrophils, monocytes, and eosinophils occasionally had an annular configuration. **Conclusions:** Reference intervals and blood cell morphology obtained in this study will be useful for the evaluation of laboratory data from ill animals and assist with population health monitoring of western barred bandicoots. (*Vet Clin Pathol.* 2007;36:XXX–XXX)

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**Key Words:** Hematology, ultrastructure, marsupial, Peramelidae, reference interval

The western barred bandicoot (*Perameles bougainville*) (Figure 1) is an endangered Australian peramelid marsupial species whose natural range is now limited to Bernier Island and Dorre Island in the Shark Bay region of Western Australia.<sup>1,2</sup> Historically, this bandicoot species occurred across the southern arid mainland of Australia but was extirpated from its former

mainland range because of habitat destruction, competition with introduced species (eg, the European rabbit, *Oryctolagus cuniculus*), and predation by introduced carnivores (eg, the red fox, *Vulpes vulpes*).<sup>3</sup> Reintroduction and captive breeding programs have been impeded by a debilitating papillomatosis and carcinomatosis syndrome.<sup>4</sup> Examinations of clinically normal and wart-affected western barred bandicoots have included hematologic analyses of peripheral blood; however, interpretation of these data has been hindered by the absence of species-specific reference intervals. The purpose of this study was to establish hematologic reference values and define morphologic features of blood cells of healthy *P bougainville* that can be used to guide clinical interpretation of laboratory data from ill animals and assist with population health monitoring.

## **Materials and Methods**

### Animals and blood samples

Samples were obtained from 3 captive populations of *P bougainville* on the Western Australian mainland: Heirisson Prong (26°02'S, 113°22'E), Dryandra Woodland (32°45'S, 116°56'E), and Kanyana Wildlife Rehabilitation Centre (31°57'S, 116°03'E). Heirisson Prong was visited in October 2005 and May 2006, Dryandra Woodland was visited in April, July and December 2005 and April 2006 and Kanyana Wildlife Rehabilitation Centre was visited in June and October 2005 and February 2006. Trapping, handling and sampling activities were conducted with the permission of the Murdoch University Animal Ethics Committee (R1115/05).

In total, 47 bandicoots (27 males and 20 females) were sampled from Heirisson Prong (n = 28; 19 males, 9 females), Dryandra Woodland (n = 13; 5 males, 8 females), and Kanyana

Wildlife Rehabilitation Centre (n = 6; 3 males, 3 females). Six females (1 from Dryandra Woodland, 5 from Heirisson Prong) were lactating. All of the animals were adults, with the exception of 1 female and 2 male subadults, distinguishable by their size, pes and head lengths, the virginal pouch of the female and scrotal dimensions in the males. Nine adult animals (5 males and 4 females from Dryandra Woodland and Kanyana Wildlife Rehabilitation Centre) were sampled on 2 or 3 occasions, with at least 3 months between consecutive blood collections; these samples were included in the analysis, for a total of 56 samples.

Western barred bandicoots were trapped at night by using baited Sheffield traps or were hand caught, as appropriate. Examinations were conducted in the morning, with the bandicoots under general anesthesia induced and maintained with isoflurane (Isorane; Baxter Healthcare, Old Toongabbie, NSW, Australia). A thorough clinical examination was performed to assess health status, and whole blood was collected by jugular venipuncture by using a 25-gauge needle. Approximately 250  $\mu\text{L}$  of blood was decanted into a 500  $\mu\text{L}$  collection tube (Microtainer, Becton-Dickinson, North Ryde, NSW, Australia) and mixed thoroughly with the dry EDTA anticoagulant.

#### Hematologic analyses

Samples from Dryandra Woodland and Heirisson Prong were refrigerated for up to 72 hours before analysis while samples from Kanyana Wildlife Rehabilitation Centre were submitted within a few hours of collection for analysis with an ADVIA-120 hematology analyzer and multispecies software using the canine setting (Bayer Diagnostics Division, Tarrytown, NY, USA). Analytes included total WBC concentration, RBC concentration, and hemoglobin (Hgb) concentrations, HCT, MCV, MCH, and MCHC.

Blood smears were made from EDTA-anticoagulated whole blood within 2 hours of collection, air-dried, and stained using a Hema-tek® Slide Stainer and Hema-tek® Wright's-Giemsa stain (Ames Company, Miles Laboratories, Victoria, Australia). The blood smears were examined by using light microscopy to determine differential leukocyte concentrations (based on counting 100 WBCs) and polychromatophilic RBC counts (based on counting 1000 RBCs); check for the presence of parasitic organisms; and assess RBC, platelet, and WBC morphology.

The total plasma protein concentration was determined by refractometry using a Reichert TS Meter (Reichert Scientific Instruments, Buffalo, NY, USA). Plasma protein was measured after centrifugation of a capillary tube filled with EDTA-anticoagulated whole blood for determination of PCV. A second centrifuged capillary tube was assessed for fibrinogen concentration by using the heat precipitation method.<sup>5</sup> (Note: sample numbers are indicated in the table).

#### Scanning and transmission electron microscopy

Approximately 500 µL of EDTA-anticoagulated whole blood was centrifuged at 500g for 10 minutes. The plasma was removed, replaced with 5% glutaraldehyde in Sorensen phosphate buffer, and refrigerated overnight. After this initial fixation, the buffy coat was harvested and post-fixed in Dalton chrome osmic acid; dehydrated through graded alcohols; transferred into propylene oxide, then propylene oxide/epon 812; and embedded in pure epon 812 (reagents were from TAAB Laboratories Equipment Ltd, Reading, Berkshire, UK). Ultrathin sections were cut, mounted on grids, stained with lead citrate and uranyl acetate, and viewed with a Philips CM 100 BioTwin transmission electron microscope (Philips; Eindhoven, Netherlands). Whole blood was fixed as described above, dehydrated through graded alcohols, dropped on a stub, and sputter-

coated with gold for visualization with a Philips XL 20 scanning electron microscope (Philips; Eindhoven, Netherlands).

### Statistical analysis

The D'Agostino-Pearson test was used to check the data for conformation to the Gaussian distribution, and the Levene test was used to assess homogeneity of variances. One-way ANOVA or Student's *t*-test was used to compare the means of data sets with homogeneous variances and parametric distributions. The Kruskal-Wallis test or Mann-Whitney *U*-test was used to compare data sets with homogeneous variances but nonparametric distributions. When data sets failed the Levene test, data were transformed by using the logarithm, square root, or reciprocal of the raw data prior to analysis. The effect of geographic location, sex, lactation status, and age was investigated for each reported analyte by using SPSS software (SPSS, version 14.0.0, Chicago, IL, USA). Statistical significance was set at  $P < .05$ .

For analytes whose data sets or transformed data sets conformed to a parametric distribution and were not significantly influenced by geographic location, sex, lactation status, or age, a Gaussian tolerance interval was calculated that had a probability of .90 of containing 95% of the population.<sup>6</sup> The lower and upper interval limits  $L_1$  and  $L_2$  are defined thus:  $L_1 = \text{sample mean} - ks$  and  $L_2 = \text{sample mean} + ks$ , where  $k = 2.252$  (for  $n = 59$ ) and  $2.276$  (for  $n = 52$ ) and  $s = [\sum(x_i - \text{sample mean})^2/n-1]^{1/2}$ .

### Results

Reference intervals and the effect of geographic location, sex, lactation status, and age

The data distribution and type of transformation was tabulated for all hematology analytes (Table 1). Because most samples had clumped platelets in blood smears, platelet counts were not reported. Hemoparasites were not observed in any samples.

Statistically significant differences were observed in some analytes in western barred bandicoots at different geographic locations. Statistically significant differences were also observed among males, females, and lactating females for MCV ( $P = .028$ ), MCH ( $P < .001$ ), MCHC ( $P = .002$ ), and fibrinogen ( $P = .019$ ) concentration (ANOVA, data not shown), however these differences were negligible from a clinical perspective. A statistically and potentially clinically significant difference was observed between adults and subadults in the percentage of polychromatophilic RBCs with subadults having  $1.0 \pm 0.20\%$  ( $n=3$ ) and adults having  $0.48 \pm 0.3\%$  ( $n=56$ ), ( $P = .004$ , Student's  $t$  test). For those analytes that did not differ significantly based on geographic location, sex, or age, Gaussian tolerance intervals were calculated (Table 1).

#### Erythrocyte morphology

Erythrocytes were anucleated, eosinophilic, shallow biconcave discocytes (Figure 2). Howell-Jolly bodies, anisocytosis, and polychromasia were occasionally seen. A few echinocytes, and sporadic stomatocytes, torocytes, knizocytes, and schistocytes were observed. Nucleated erythrocytes were very rarely observed.

#### Leukocyte morphology

Neutrophils and lymphocytes were the most numerous leukocytes, with fewer eosinophils and monocytes, and rare basophils (Table 1). Neutrophils (Figure 3) had 3 to 7 lobes of often tortuously segmented and densely clumped nuclear chromatin. The almost colorless cytoplasm



contained weakly-staining granules (Figure 3a). Occasionally, mature neutrophil nuclei were hyposegmented or had an annular configuration (Figure 3b). Immature neutrophils had band-shaped or annular nuclei, and basophilic cytoplasm with scattered azurophilic granules.

Ultrastructurally, neutrophil nuclei typically were multilobulated and composed of more hetero- than euchromatin. The cytoplasm contained several organelles, including rough endoplasmic reticulum, Golgi body, mitochondria, a few small vacuoles, and numerous membrane-bound granules of at least 2 types. The most numerous granule type was ovoid to elongate in longitudinal section, round in cross-section, and electron-dense (secondary granules). Round to ovoid and less electron-dense granules consistent with primary granules also populated the cytoplasm but were far fewer in number (Figure 3c).

Lymphocytes had central, round, ovoid or indented nuclei surrounded by light basophilic cytoplasm. Small lymphocytes had high nuclear to cytoplasmic (N:C) ratios, scant cytoplasm, and small nuclei with dense chromatin; whereas medium and large lymphocytes had lower N:C ratios, more abundant basophilic cytoplasm, and larger, less densely staining nuclei with more dispersed chromatin. Ultrastructurally, lymphocytes had round nuclei with moderately condensed chromatin rimmed by cytoplasm that contained several mitochondria.

Monocytes (Figure 4) were the largest leukocytes and often had an irregular cell shape. Nuclei were usually horseshoe-shaped or irregular, had reticular chromatin, and were surrounded by abundant mildly basophilic cytoplasm (Figure 4a). Monocyte nuclei frequently had an annular configuration (Figure 4b). Ultrastructurally, monocytes had indented or irregular nuclei, with more abundant euchromatin than neutrophils, and a cytoplasm rich in variably sized vacuoles, mitochondria, ribosomes, and moderately electron-dense primary granules. There were numerous fine projections of the plasma membrane, some fusing to form phago- or pinocytotic

vesicles (Figure 4c). Eosinophils (Figure 5) had 2–4 nuclear lobes with densely clumped chromatin and a moderate amount of pale basophilic cytoplasm, with innumerable ovoid brightly eosinophilic granules scattered throughout (Figure 5a). Some eosinophil nuclei had an annular configuration (Figure 5b). In transmission electron micrographs, eosinophil nuclei were multilobulated and composed of hetero- and euchromatin. Eosinophil granules were large, ovoid in longitudinal section, and circular in cross-section, with homogeneous electron density; they were noticeably larger than neutrophil granules. No distinct crystalline structures were observed within eosinophil granules; however, 16 of 124 granules from 4 photographed eosinophils (12.9%) had small, round, membrane invaginations (Figure 5c).

Basophils typically had 2 nuclear lobes of densely clumped chromatin, a moderate amount of faintly basophilic cytoplasm that contained numerous variably-sized round to irregular, intensely basophilic granules that frequently obscured the nucleus. Basophils were not identified on transmission electron microscopy.

#### Platelet morphology

Platelets varied markedly in size, from 1 to 6  $\mu\text{m}$ , lacked a nucleus, and had slightly basophilic cytoplasm with many azurophilic granules.

## Discussion

The light microscopic appearance of erythrocytes and leukocytes in this study was similar to that previously reported for *P bougainville*<sup>7</sup>, and ultrastructural features of leukocytes were comparable to those described by Clark<sup>7</sup> for other marsupial species. All leukocyte types

typically present in mammalian blood smears were found in blood smears from healthy *P bougainville*. Total leukocyte concentrations in peramelemorphs such as bilbies (*Macrotis lagotis*), northern brown bandicoots (*Isoodon macrourus*) southern brown bandicoots (*Isoodon obesulus*) and eastern barred bandicoots (*Perameles gunnii*) tend to be low compared with other mammalian orders<sup>7</sup>, and *P bougainville* was no exception. Hematologic data reported for the northern brown bandicoot (*I macrourus*) and eastern barred bandicoot (*P gunnii*)<sup>8</sup> were similar to those obtained for *P bougainville*. Our findings for *P bougainville* were also similar to those reported for southern brown bandicoots (*I obesulus*) from Western Australia.<sup>9</sup> Samples from captive and wild populations of juvenile northern brown bandicoots (*I macrourus*)<sup>10</sup> had comparable HCTs with those we obtained for *P bougainville*; however, total leukocyte concentrations were approximately double those we obtained in this study, with very high percentages of circulating lymphocytes and low percentages of circulating neutrophils.

Hematologic data from bilbies (*M lagotis*)<sup>11</sup> included higher values for HCT, MCV, MCH, RBC, Hgb, and total WBC concentrations than those we obtained for *P bougainville*. Differences in levels of stress, sedation and anesthetic protocol, site of venipuncture, age, presence of subclinical disease, and methods of analysis, as well as true differences between the 2 peramelemorph species may explain these dissimilarities.

The complex interplay between genetics, environment, nutrition, age, sex, and social structure may account for the differences we observed in some hematologic results between colonies of *P bougainville* at different geographic locations, as previously reported for Parma wallabies (*Macropus parma*).<sup>12</sup> Nutrition, environment, and social structure are likely to vary considerably between the 3 study sites and, therefore, may be the most important of these factors in terms of the current study. Western barred bandicoots at the 3 study sites are all descendents

of *P bougainville* translocated from Bernier Island or Dorre Islands and, therefore, are closely related.

One potential limitation of this study was the length of time (72 hours) between sample collection and analysis. Results of experiments with the western grey kangaroo (*Macropus fuliginosus*) indicate that delays of up to 108 hours between blood sample collection and processing have minimal effects on RBC parameters if samples are refrigerated at 4°C.<sup>13</sup> A transient mild decrease in RBC and Hgb concentrations was noted in western grey kangaroo blood samples refrigerated for up to 48 hours, after which time these values returned to normal.<sup>13</sup> The effect of protracted refrigerated storage on total WBC concentration is more difficult to predict; however, the total WBC concentrations from the 3 geographic locations were all approximately equal, despite discrepancies in storage time. In our study, blood smears were made at the time of sample collection, therefore, cell morphology and WBC differential counts could be accurately assessed. Underfilling of collection tubes that contained dry EDTA anticoagulant may have resulted in echinocytes observed because of RBC crenation.<sup>14</sup> Excess EDTA also can artifactually increase total plasma protein concentration, as measured by refractometry. Half-filling the EDTA tubes used in this study appeared to induce an overestimate of approximately 3 g/L in the total plasma protein concentration when assessed by refractometry compared with the biuret method for plasma protein concentration determination (unpub. obs.).

Platelet aggregation, observed in almost all samples, may lead to spuriously decreased platelet concentrations and increased mean platelet volumes using automated analyzers; thus the results were not reported in this study. Platelet aggregation may have resulted from prolonged storage in EDTA.

Despite these limitations, to our knowledge, this is the most comprehensive study of the

hematology of *P bougainville* documented thus far in the scientific literature. The population sampled is approximately 10% of the estimated captive population, and comprises 1%–2% of all western barred bandicoots thought to be alive. The reference values and morphologic descriptions in this study characterize the expected hematologic findings in healthy western barred bandicoots and can be used to inform clinical decision-making and population health monitoring.

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## References

1. Friend JA, Burbidge AA. Western barred bandicoot. In: Strahan R, ed. *The Mammals of Australia*. 2nd ed. Frenchs Forest, NSW, Australia: Reed New Holland; 2002:178–180.
2. International Union for the Conservation of Nature and Natural Resources (IUCN) List of Threatened Species. Available at: <http://www.redlist.org/search/details.php?species=16569>. Accessed October 4, 2007.
3. Tyndale-Biscoe H. *Life of Marsupials*. Melbourne, Australia: CSIRO Publishing; 2005:165–181.
4. O'Hara AJ, Warren KS, Swan RA et al. Cutaneous papillomatosis and carcinomatosis in the endangered western barred bandicoot (*Perameles bougainville*) [abstract]. *Vet Dermatol*. 2004;15(Suppl 1):25.
5. Jain NC. Hematologic techniques. In: *Schalm's Veterinary Hematology*. 4th ed. Philadelphia, PA: Lea & Febiger; 1986:58–59.
6. Lumsden JH, Mullen K. On establishing reference values. *Can J Comp Med*. 1978;42:293–301.
7. Clark P. *Haematology of Australian Mammals*. Melbourne, Australia: CSIRO Publishing; 2004.
8. Parsons RS, Atwood J, Guiler ER, Heddle RWL. Comparative studies on the blood of monotremes and marsupials—I. Haematology. *Comp Biochem Physiol*. 1971;39B:203–208.
9. Wicks RM, Clark P. Clinical haematology of the southern brown bandicoot (*Isoodon obesulus*). *Comp Clin Pathol*. 2005;14:56–60.
10. Gemmell RT, Cepon G, Green PE, Stewart NP. Some effects of tick infestation on juvenile northern brown bandicoots (*Isoodon macrourus*). *J Wildl Dis*. 1991;27:269–275.

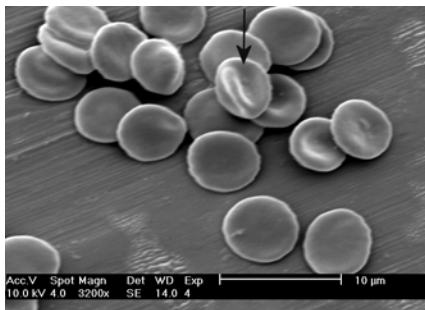
11. Agar NS, Godwin IR. Red cell metabolism in the brown antechinus, the bilby and the rufous hare-wallaby. *Aust J Zool.* 1991;39:681–687.
12. Clark P, Holz P, Booth R, et al. Haematological characteristics of captive Parma wallabies (*Macropus parma*). *Comp Clin Pathol.* 2003;12:11–16.
13. Hulme-Moir KL, Clark P, Spencer PBS. Effects of temperature and duration of sample storage on the haematological characteristics of western grey kangaroos (*Macropus fuliginosus*). *Aust Vet J.* 2006;84:143–147.
14. Brockus CW, Andreasen CB. Erythrocytes. In: *Duncan and Prasse's Veterinary Laboratory Medicine Clinical Pathology.* 4th ed. Ames, IA: Iowa State Press; 2003:3-45.

**Figure 1.** Clinically healthy adult western barred bandicoot (*Perameles bougainville*).

Photograph courtesy of Linda Reinhold.



**Figure 2.** Scanning electron micrograph of erythrocytes of *Perameles bougainville*. A knizocyte can be seen (arrow).

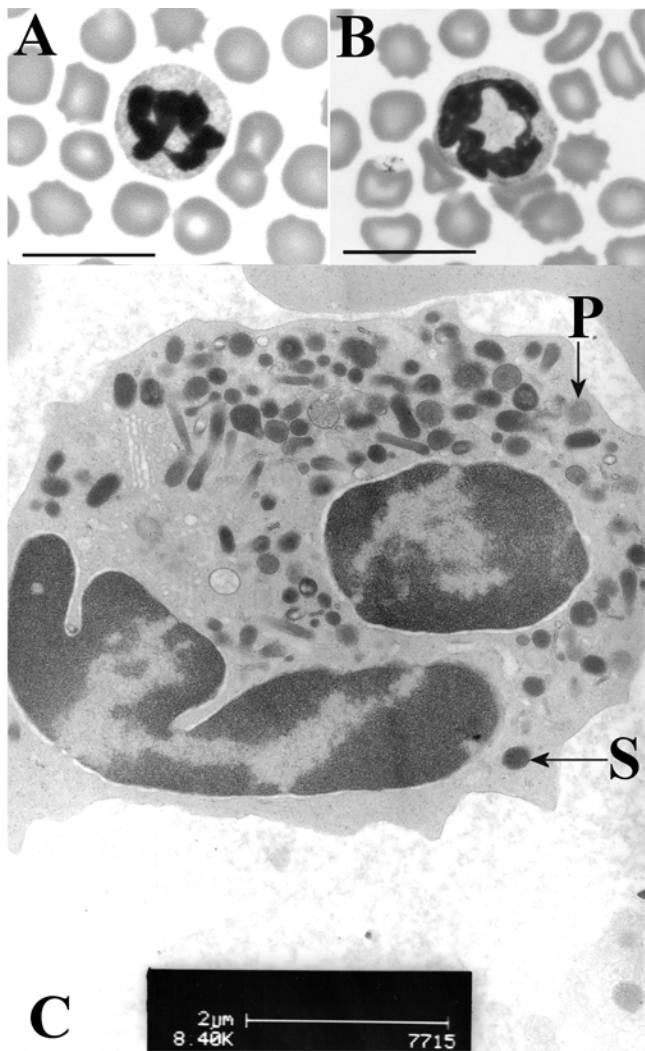


**Figure 3.** Neutrophils from *Perameles bougainville*. (A) A typical segmented neutrophil.

Wright's-Giemsa, bar = 10 µm. (B) A segmented neutrophil with an annular nuclear



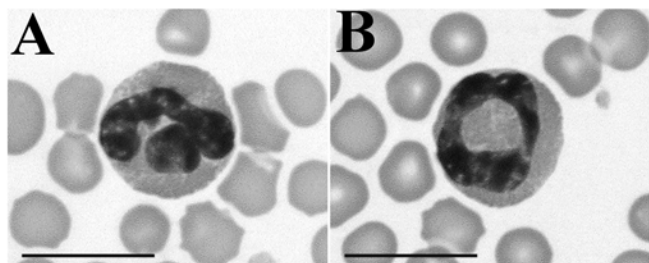
configuration. Wright's-Giemsa, bar = 10  $\mu\text{m}$ . (C) Transmission electron micrograph of a segmented neutrophil. The neutrophil nucleus appears divided into 2 parts by the plane of section. Primary (P) and secondary (S) granules can be seen in the cytoplasm. Lead citrate and uranyl acetate stains.



**Figure 4.** Monocytes from *Perameles bougainville*. (A) A typical monocyte with a large horseshoe-shaped nucleus and abundant cytoplasm. Wright's-Giemsa, bar = 10  $\mu\text{m}$ . (B) A monocyte with an annular nuclear configuration. Wright's-Giemsa, bar = 10  $\mu\text{m}$ . (C) Transmission electron micrograph of a typical monocyte with an indented nucleus and numerous projections and invaginations of the plasma membrane. Lead citrate and uranyl acetate stains.



**Figure 5.** Eosinophils of *Perameles bougainville*. (A) A typical eosinophil with a lobulated nucleus and abundant eosinophilic cytoplasmic granules. Wright's-Giemsa, bar = 10 μm. (B) An eosinophil with an annular nuclear configuration. Wright's-Giemsa, bar = 10 μm. (C) Transmission electron micrograph of a typical eosinophil. Some cytoplasmic granules have a round membrane invagination (arrow). Lead citrate and uranyl acetate stains.



**Table 1.** Hematology and plasma protein values (minimum-maximum) for 3 captive populations of clinically healthy western barred bandicoots (*Perameles bougainville*) in Western Australia.

Analyte	Heirisson Prong (n = 28)	Dryandra Woodland (n = 20)	Kanyana Wildlife Rehabilitation Centre (n = 22)	Data Distribution or Transformation	P Value*	Reference Interval† (n = 59)
RBCs ( $\times 10^{12}/L$ )	6.24–7.83	5.57–7.65	5.68–7.51	Normal	.006	—
Hemoglobin (g/L)	125–153	118–167	127–162	Reciprocal	.778	122–165
HCT (L/L)	0.38–0.47	0.36–0.48	0.39–0.50	Normal	.327	0.36–0.49
MCV (fL)	58.8–63.1	60.3–66.7	60.3–67.9		—	—
MCH (pg)	19.1–20.7	20.3–22.2	20.2–22.5		—	—
MCHC (g/L)	315–336	315–345	316–344	Normal	.003	—
Polychromatophilic RBCs (%)	0.1–0.9	0–1.2	0.3–1.3	Normal	<.001	—
Total WBCs ( $\times 10^9/L$ )	3.6–12.4	2.7–11.4	3.4–13.2	Logarithm	.305	2.9–14.9
Neutrophils ( $\times 10^9/L$ )	2.25–10.3	0.7–8.8	1.1–3.7	Normal	<.001	—
Lymphocytes ( $\times 10^9/L$ )	0.5–3.2	0.5–5.3	1.5–10.6	Logarithm	<.001	—
Monocytes ( $\times 10^9/L$ )	0–0.6	0–0.5	0–0.5	Square root	.676	0–0.6
Eosinophils ( $\times 10^9/L$ )	0–0.6	0–0.5	0–1.1	Square root	.172	0–0.9
Basophils ( $\times 10^9/L$ )	0–0.1	0–0.1	0–0.1		—	—
Total plasma protein (g/L)	50–62	46–62‡	51–61‡	Normal	.562	47–63‡
Fibrinogen (g/L)	<1–4	1–4‡	1–2‡	Square root	.020	—

\*P values obtained by 1-way ANOVA for results from western barred bandicoots at different geographic locations.

†Gaussian tolerance limits calculated on normally distributed or transformed data for all sites combined. See text for the details of the calculation.

‡Data were obtained from fewer samples: plasma protein concentration at Dryandra (n = 17), Kanyana (n = 7), and reference interval (n = 52); fibrinogen concentration at Dryandra (n = 16) and Kanyana (n = 3).