Haematological and serum biochemical results for anaesthetised northern brown bandicoots (*Isoodon macrourus*) in south east Queensland.

RH: Northern brown bandicoot reference intervals.

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Abstract

Bandicoots are terrestrial marsupials, endemic to Australia and New Guinea. Despite drastic declines in several bandicoot species since European settlement, the northern brown bandicoot (*Isoodon macrourus*) remains common in many areas of Australia. It inhabits native environments as well as anthropogenic landscapes such as suburban gardens, parks and agricultural properties. This study presents comprehensive haematologic and serum biochemical reference intervals for 40 clinically healthy, wild-caught, adult northern brown bandicoots in south east Queensland, Australia. The bloodwork profile of a single animal with chronic prostatic abscessation highlights that haematology and clinical chemistry can provide useful biomarkers for identifying clinical disease in bandicoots. Haematologic values, compared with those reported for southern brown bandicoots (*Isoodon obesulus*), demonstrate significant differences in mean values for leukocytes, neutrophils and haematocrit. Comparisons of haematologic and biochemical values between sexes of the northern brown bandicoot revealed significant differences for eosinophils, alkaline phosphatase (ALP) and total bilirubin. At the individual animal level, the reference intervals established in this study are a guide for monitoring health and disease status, however they also have much wider applications in population health, ecological research and public health.

Keywords: bandicoot, biochemistry, clinical pathology, hematology, *Isoodon*

Despite several species of bandicoot faring poorly since European settlement, the northern brown bandicoot (*Isoodon macrourus*) remains common in many areas of Australia. They have adapted to changing environments and are found inhabiting many anthropogenic landscapes. Although the northern brown bandicoot is considered a common species, very little research into the health of this bandicoot has been conducted. Gemmell, Cepon, Green and Stewart [1] reported on a limited number of haematology parameters from 15 wild caught northern brown bandicoots. Wicks and Clark [2] subsequently provided a thorough guide to haematology in the southern brown bandicoot. However, to date, comprehensive haematologic and biochemical reference ranges for the northern brown

bandicoot have not been published. This study provides haematologic and biochemical reference intervals for clinically healthy, wild-caught, northern brown bandicoots in south east Queensland.

While haematologic and serum biochemical reference intervals are vital for assessing the health and disease status of wildlife, they also have wider applications in ecological research and the study of infectious disease dynamics within wildlife populations [3].

All work was conducted under the University of Queensland Animal Ethics Committee Permit SAFS/036/18, and the Department of Environment and Science, Scientific Purposes Permit WA0007547. Northern brown bandicoots were trapped on 10 study sites in south east Queensland from October 2018 to September 2019. Small cage traps were baited with peanut butter on bread. Trapped bandicoots were transported in calico bags to the veterinary clinic at the Hidden Vale Wildlife Centre, Grandchester. Each animal was anaesthetised using isoflurane in oxygen delivered by facemask. A brief physical examination was performed, pes length measured, presence and number of pouch young recorded in females, and scrotal width measured in males. Criteria for classification as an adult were as follows: dark pigmentation of scrotum and testis length at least 20 mm for males [4]; and for females the pouch contained elongated teats or suckling young; where these features were absent a weight threshold of 800 g was applied[4]. Molar tooth wear was also assessed and graded on a 4 point scale to further assist in aging adults. Venepuncture was performed from the jugular vein within 15 minutes of anaesthetic induction. All animals were monitored until fully awake post anaesthetic; they were then held in the transport bags in cages at the veterinary clinic until they were released at the same site at dusk on the day of capture. Only animals that were clinically normal on physical examination had data used for reference interval development.

A blood sample not exceeding 1% of the total body weight was taken from the jugular vein [5]. Blood was transferred into 0.5 ml EDTA tubes (Greiner Bio-One, 450530, Kremsmünster, Austria) and 1.3 ml serum blood tubes (Sarstedt AG & Co, 41.1392.105, N¨umbrecht, Germany) and a thin blood film prepared. Samples were kept at 4°C and transferred to the University of Queensland School of Veterinary Science within 4 hours of collection. Haematologic analysis was performed on EDTA whole blood using a Cell Dyn 3700 haematology analyser (Abbott laboratories Chicago IL, USA). Manual packed cell volume and total protein was also performed on the EDTA whole blood.

Biochemical analysis was performed on serum samples using a Beckman Coulter AU480 biochemistry analyser (Beckman coulter Inc, CA, USA). Air dried blood smears were stained with Wrights Giemesa stain using a Hama-Tek 1000 (Siemens Healthcare, NY, USA). All analyte values are reported in standard international (SI) units.

Reference intervals for hematologic and biochemistry analytes were calculated as per the ASVCP reference interval guidelines for between 20 to 40 samples, using mean +/- 1.64 standard deviations (90% confidence interval) [6]. Outliers were eliminated from the sample population and variables that were not normally distributed were transformed using natural log. Comparisons of analytes between sexes were completed using ANOVA (XL Toolbox V7.3.2, 2019 www.xltoolbox.net). A two sample z – test for comparing two means was used to compare the data in this study to that of Wicks and Clark [2] on southern brown bandicoots.

During this study, clinically healthy male and female bandicoots (20 of each sex), were trapped. In addition, blood was collected from one adult male showing clinical signs of disease (omitted from reference interval calculation). Means, range and reference intervals for hematologic analytes are listed and compared with the southern brown bandicoot in Table 1, and biochemical data in Table 2.

Insert Table 1

Insert Table 2

Following capture and sampling, an older (molar wear score 4/4) male, northern brown bandicoot with a large prostatic abscess was humanely euthanized on welfare grounds. This individual had changes to haematological and biochemistry analytes. These included a marked leukocytosis (21.2 $x10^9$/L) characterized by left shifted neutrophilia (18.02 $x10^9$/L) with some toxic changes upon blood film review. These are consistent with severe inflammation caused by bacterial infection, highlighting the extent to which neutrophils may be elevated in this species due to an infectious process. Plasma biochemistry indicated marked hyperproteinemia (78.8 g/L) due to hyperglobulinemia (55.8 g/L) indicative of chronic inflammatory processes. Mild hypermagnesemia (1.22 mmol/L), marked hyperkalaemia (7.9 mmol/L) and marked azotaemia (27.1 mmol/L) was also demonstrated. The azotaemia was likely due to a combination of pre-renal, renal and post-renal factors. The

hypermagnesemia and hyperkalaemia suggest a renal contribution to the azotaemia might have occurred. The animal showed clinical signs of dehydration and necropsy confirmed moderate urinary obstruction due to the large abscess. The abnormalities in the blood profile of this individual were consistent with those seen in other species.

Indices of red cell mass including RBC, HGB, HCT and PCV were all lower in the northern brown bandicoots in the present study compared to southern brown bandicoots [2]. However, only HCT was statistically significant. In domestic animals, red cell indices can be variable between species and even breeds, for example higher in greyhounds compared to other dog breeds. The differences in red blood cell indices between the two bandicoot species may be a true species difference, however further analysis on a larger sample size would be required to fully explore this possibility.

Mean leukocyte and neutrophils counts were significantly different between northern brown and southern brown bandicoots. While this could be a species effect, differences in methodology between the two studies might also explain the discrepancy. The increased neutrophil, and thus leukocyte count, in the current study might represent a mild physiological manifestation related to increased cortisol (a 'stress leukogram'). Bandicoots in Wicks and Clark [2] were sampled within 'one to several hours of being captured,' sedated and sampled trap-side.In the current study, traps were set at dusk and checked at dawn and animals were transported to a veterinary clinic for sampling. Investigators should be aware that the leukogram in bandicoots is likely to be moderately labile, particularly where trapped animals are captive for variable periods.

A previous study on hematology of northern brown bandicoots highlighted a mean leukocyte count of 13.95 $x10^9$/L for 15 wild caught bandicoots [1]. Lymphocytes were the predominant leukocyte recorded, representing 85% of total leukocyte count. These are inconsistent with the current study, where mean leukocyte count was 6.26 $x10^9$/L and neutrophils were the predominant leukocyte. These differences may be due to subclinical disease in the bandicoots not detected during the earlier study. This theory was originally suggested by Wicks and Clark [2] who found that southern brown bandicoots also had significantly lower mean leukocyte count and that neutrophils were again the predominant leukocyte.

In the present study, as expected, mean values and reference intervals were generally consistent between healthy male and female northern brown bandicoots. Only 3 variables were significantly different: eosinophils, alkaline phosphatase (ALP) and total bilirubin. For all 3 analytes, mean values for females were significantly higher than those for males. Further analysis indicated no significant difference for these variables between females with pouch young compared to those without. Hemogregarine parasites consistent with *Hepatozoon* spp. were detected in 16.6% of blood films (6/36), via visual microscopy. Animals infected with *Hepatozoon* spp. had statistically higher eosinophil counts than those uninfected, and females accounted for 67% (4/6) of detections. The higher eosinophil count in females may be indicative of an increased parasite burden concomitant with immunosuppression due to pregnancy or lactation. However, this remains speculative in the present study given the small sample size, and that a complete examination of parasite burden was not undertaken.

In some mammals, ALP has been shown to increase in females in late gestation, this is one clinical possibility for the sex difference in ALP [7]. However, given the limited sample size in this study, it is possible that differences in ALP and total bilirubin between male and female animals are of no clinical significance.

It is worth noting that platelet count in the present study ranged from 33.5 - 351.0 $x10^9$/L. Blood film review for all animals with a lower than expected platelet count revealed numerous clumps of platelets. For this reason, machine calculated platelet counts should be interpreted with caution and blood film review is recommended, to ensure accurate estimation of platelet mass.

This study provides baseline haematological and biochemical data essential for monitoring the health of northern brown bandicoots. This data is valuable in the context of individual disease diagnosis as demonstrated by the diseased bandicoot, and in much broader applications such as monitoring population health to understand disease dynamics within wildlife species.

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Table 1: Hematology reference intervals, means and range for adult wild-caught northern brown bandicoots (NBB) in south east Queensland. Mean values for wild-caught southern brown bandicoots (SBB) in southern Perth taken from [2].

	NBB						SBB
					Male	Female	
	n	**Range**	**Mean (SD)**	**Reference interval**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
WBC ($x10^9$/L)*	40	2.73 - 12.1	**6.26 (1.52)**	3.13 - 12.47	6.40 (2.21)	7.18 (3.24)	**3.50 (1.40)**
NEU ($x10^9$/L)*	38	0.92 - 8.23	**3.01 (1.7)**	1.24 - 7.25	3.35 (1.71)	4.01 (2.35)	**1.34 (0.78)**
LYM ($x10^9$/L)*	39	0.41 - 6.20	1.63 (2.12)	0.47 - 5.60	2.31 (1.93)	2.19 (1.55)	1.74 (1.11)
MONO ($x10^9$/L)*	38	0 - 0.9	0.28 (2.09)	0.08 - 0.95	0.36 (0.25)	0.38 (0.23)	0.04 (0.05)
EOS ($x10^9$/L)*	39	0 - 0.82	0.24 (1.9)	0.08 - 0.68	**0.18 (0.16)**	**0.33 (0.20)**	0.31 (0.21)
BASO ($x10^9$/L)*	39	0 - 0.23	0.25 (3.98)	0.02 - 2.37	0.03 (0.04)	0.07 (0.08)	0.01 (0.01)
RBC ($x10^{12}$/L)	40	3.14 - 7.76	5.40 (1.70)	3.51 - 7.28	5.5 (1.29)	5.14 (1.25)	6.96 (0.56)
HGB (g/L)	40	58.9 - 158.0	109.1 (23.58)	70.45 - 147.79	110.6 (24.99)	104.3 (27.38)	145 (9.8)
HCT (L/L)	40	0.19 - 0.47	**0.33 (0.07)**	0.22 - 0.44	0.33 (0.07)	0.32 (0.08)	**0.43 (0.04)**
MCV (fL)	40	53 – 71	61.53 (4.17)	54.69 - 68.36	60.91 (4.48)	62.59 (4.36)	61.4 (2.3)
MCH (pg)	40	16.6 - 26.0	20.19 (1.77)	17.29 - 23.09	20.25 (2.02)	20.28 (1.68)	20.8 (1.0)
MCHC (g/L)	40	307 – 370	328.6 (14.17)	305.4 - 351.9	332.4 (15.40)	323.5 (13.72)	338 (11)
RDW %	39	14.2 - 20.6	17.27 (1.44)	14.91 - 19.63	17.91 (2.53)	17.42 (2.03)	11.0 (0.8)

PLT (x10^9/L)*	35	33.5 - 351.0	99.73 (67.22)	41.17 - 241.55	108.1 (55.86)	111.9 (80.67)	-
Man. PCV (L/L)	39	15 - 44	30.77 (6.55)	20.03 - 41.51	32.45 (6.06)	30.52 (8.13)	41 (0.4)
Protein (g/L)	39	50 - 69	58.31 (4.73)	50.55 - 66.06	57.65 (6.32)	58.90 (5.81)	59 (5.3)

*Natural log transformed due to skew of data. Bold values indicate a significant difference between species or gender.

Table 2: Biochemistry reference intervals, means and range for adult wild-caught northern brown bandicoots in south east Queensland.

	n	Mean	Range	Reference Range	Male	Female
Albumin (g/L)	37	29.87	23.5 - 36.1	24.31 - 35.43	30.41 (3.87)	29.26 (3.99)
ALP (U/L)*	37	210.4	71.5 - 630.2	73.03 - 606.19	**366.3 (282.9)**	**199.7 (131.2)**
ALT (U/L)*	38	60.09	8.5 - 175.0	18.11 - 199.34	86.66 (39.56)	69.15 (52.00)
AST (U/L)*	38	40.96	7.5 - 100.3	14.42 - 116.32	52.31 (27.13)	50.59 (30.53)
Bicarbonate (mmol/L)	38	18.57	13 - 25.2	13.53 - 23.60	18.33 (5.16)	17.88 (2.85)
Calcium (mmol/L)	38	2.09	1.63 - 2.46	1.73 - 2.43	2.12 (0.25)	2.03 (0.22)
Cholesterol (mmol/L)	39	2.30	1.52 - 3.35	1.59 - 3.00	2.20 (0.45)	2.39 (0.42)
CK (U/L)*	34	242.55	11.1 - 1247.1	36.1 - 1629.4	625.9 (567.3)	438.8 (409.25)
Creatinine (umol/L)*	36	44.67	12.9 - 81.5	23.92 - 83.41	56.79 (23.36)	44.74 (15.91)
GGT (U/L)*	23	0.93	0 - 2.1	0.39 - 2.16	0.83 (0.56)	1.27 (0.56)
Glucose (mmol/L)	36	4.47	1.64 - 6.97	2.57 - 6.36	4.92 (1.83)	5.16 (2.90)
Phosphate (mmol/L)	38	2.21	1.37 - 3.55	1.34 - 3.07	2.52 (0.85)	2.09 (0.51)
Magnesium (mmol/L)	39	0.78	0.51 - 0.99	0.59 - 0.97	0.78 (0.13)	0.78 (0.10)
Total bilirubin (umol/L)*	36	3.38	0.2 - 10.0	0.95 - 11.87	**3.55 (2.23)**	**5.57 (3.57)**
Total protein (g/L)	38	50.96	42.0 - 61.9	42.75 - 59.16	50.72 (5.24)	52.27 (7.02)

Triglycerides (mmol/L)	37	1.29	0.6 - 2.39	0.54 - 2.03	1.48 (0.81)	1.32 (0.46)
Urea (mmol/L)	38	10.37	6.69 - 15.68	6.5 - 14.2	10.34 (2.27)	10.90 (2.84)
Globulins (g/L)	37	21.26	14.0 - 34.1	13.57 - 28.96	20.16 (5.79)	23.01 (6.17)
Sodium (mmol/L)	38	140.34	127 -155	129.8 - 150.86	139.6 (9.61)	139.5 (6.12)
Potassium (mmol/L)	36	4.49	3.13 - 6.02	3.4 - 5.56	4.77 (1.00)	4.59 (0.81)
Chlorine (mmol/L)	38	104.74	96 – 115	96.36 - 113.10	103.3 (6.92)	105.0 (5.12)

*Natural log transformed due to skew of data