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HEMATOLOGIC AND SERUM BIOCHEMICAL REFERENCE VALUES FOR FREE-RANGING NORTHERN HAIRY-NOSED WOMBATS

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ABSTRACT: Hematologic and serum biochemistry values were determined for 31 adult (21 male and 10 female) and four subadult male northern hairy-nosed wombats (*Lasiorninus krefftii*) from the only existing population in Epping Forest National Park, Australia. Blood samples were obtained from free-ranging northern hairy-nosed wombats during trapping for population census and health and reproductive assessment in 1999. Hematologic and biochemical values were compared between adult males and adult females, and between adult and subadult wombats. Values were also compared with those previously published for southern hairy-nosed (*Lasiorninus latifrons*) and common (*Vombatus ursinus*) wombats. The values from this study were used to create reference intervals, and they make up the first comprehensive hematologic and biochemical study for this highly endangered species.

Key words: Biochemistry, clinical pathology, hematology, *Lasiorninus krefftii*, wombat.

INTRODUCTION

The northern hairy-nosed wombat (*Lasiorninus krefftii*) is one of the world's rarest mammals; it is listed as "critically endangered" by the International Union for the Conservation of Nature and Natural Resources (IUCN) Species Survival Commission (2001). The population, which is estimated at only 115 individuals, lives free-ranging in a 500-ha area of Epping Forest National Park, near Clermont in central Queensland, Australia (Horsup, 2004). There are no northern hairy-nosed wombats in captivity. The range of this population has contracted significantly in the past 50 yr. Hairy-nosed wombats are mostly nocturnal, and they inhabit native perennial grasslands and open woodlands in a semiarid, drought-prone environment (Johnson, 1991a).

Threats to the northern hairy-nosed wombat include predation by dingoes (*Canis familiaris dingo*) (Banks et al., 2003a); wildfire, drought, loss of genetic diversity, a gender bias to males (Taylor et al., 1994); grazing competition from eastern grey kangaroos (*Macropus giganteus*) (Woolnough and Johnson, 2000); disease (O'Brien and Evermann, 1988; Gerhardt et al., 2000); loss of habitat through land

clearing, and invasion of native pasture by introduced buffel grass (*Cenchrus ciliaris*) (Horsup, 2004).

The northern hairy-nosed wombat population is managed by the Queensland Parks and Wildlife Service, through the northern hairy-nosed wombat recovery program. Burrow activity observations and noninvasive hair collection for DNA analysis provide assessments of population dynamics (Banks et al., 2003b). In addition, trapping of animals is carried out approximately every 3 yr (Horsup, 2004). Due to the small size of the population and the reclusive nature of northern hairy-nosed wombats, which makes them difficult to capture, there have been limited opportunities to collect health data or to investigate disease in this species.

An extended trapping exercise was conducted in 1999, for the purposes of population census, collection of morphometric data, and reproductive and health assessments. This trapping exercise provided a valuable opportunity to collect blood samples from a large proportion of the clinically healthy population and to develop reference intervals for hematologic and serum biochemical tests that could be used in future disease investigations.

MATERIALS AND METHODS

Live trapping of northern hairy-nosed wombats was carried out from May through September 1999 at Epping Forest National Park, a 3,300-ha scientific reserve, northwest of Clermont in central Queensland, Australia (22°21'S, 146°42'E). Wombats were captured in steel traps as described previously (Hoyle et al., 1995). Wombats were anesthetised using tiletamine/zolazepam (Zoletil 100, Virbac Australia Pty Ltd., Peakhurst, New South Wales, Australia); 2–3 mg/kg by intramuscular hand injection (Evans et al., 1998). Supplemental anesthesia, when required, was provided by isoflurane (Laser Animal Health Isoflurane, Pharmachem, Salisbury, Queensland, Australia) in oxygen, delivered by a portable precision vaporizer. Clinical health was judged on the basis of subjective assessment of findings during physical examination. For the purposes of this study, subadult wombats were classified as those with a body weight <19 kg.

Up to 26 ml of blood was collected from the cephalic vein from a total of 35 wombats: four subadult males, 21 adult males, and 10 adult females. The large numbers of males sampled compared with females reflects the sex skew of the population at the time of this study (Horsup, 1998).

Blood samples were placed into ethylenediaminetetraacetic acid (EDTA) and serum separator tubes (Vacutainer, BD Biosciences, Franklin Lakes, New Jersey, USA). Serum tubes were refrigerated, allowed to clot for 1–6 hr, and then centrifuged. Serum was frozen at –20 C, and EDTA samples were refrigerated. Blood smears were made from EDTA blood within 3 hr of collection and air-dried. Serum and EDTA samples (on ice) and blood smears were shipped to a commercial laboratory for processing. Due to the remote location of the field site, samples were processed up to 72 hr after collection.

Hematologic and biochemical analyses were performed by Veterinary Pathology Services, Brisbane, Australia. Hematologic analysis was performed on EDTA whole blood using a Cell-Dyn 3500 automated hematology analyzer (Abbott Diagnostics, Abbott Park, Illinois, USA). Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin, packed cell volume, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were measured. The WBC differential and number of nucleated red cells/100 WBCs was determined manually by blood film examination. Smears were examined for estimation of

platelet numbers and assessment of platelet, WBC, and RBC morphology.

Biochemical analyses were performed on serum using an Olympus AU 400 analytical chemistry analyzer (Olympus Optical Company, Tokyo, Japan). Alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine transaminase (ALT), creatine kinase (CK), total bilirubin, urea, creatinine, cholesterol, calcium, phosphorus, sodium, chloride, potassium, bicarbonate, glucose, albumin, globulin, and total serum protein were analyzed.

All results were entered into a computerized database (Excel, Microsoft Office 95, Microsoft Corporation, Redmond, Washington, USA) and checked for accuracy. Most parameters seemed to have normal distribution, but no assumptions of normalcy were made. Data were examined for outliers. Several obvious outlying results (total bilirubin, AST, ALT, and CK) were found from one adult female, exhibiting signs consistent with myopathy. These values were excluded from the data set.

Results from individuals were initially grouped according to gender: males ($n=25$, including subadults) and females ($n=10$), for analysis. Results were also grouped according to age class: subadults ($n=4$) and adults ($n=31$).

Means, variances, and standard deviations for each data group were calculated using the Excel computer program. We used *t*-tests to compare means and to evaluate significant differences between values for male and female wombats, and adult male and subadult male wombats. Statistical significance was assessed at a level of $P<0.05$.

Suggested reference intervals for adult northern hairy-nosed wombats were created by ranking data and using the central 90th percentile nonparametric analysis (Duncan et al., 1994; Linnet, 2000). Due to the small sample size, reference intervals for subadults are stated as minimum and maximum values.

RESULTS

Thirty-five blood samples were obtained from 25 male and 10 female northern hairy-nosed wombats. All animals, with the exception of two, were judged to be healthy on the basis of clinical examination. One subadult male weighing 9.2 kg was judged to be underweight and possibly malnourished. One adult female was presumed to be suffering from myopathy and capture stress.

TABLE 1. Mean \pm SD and reference intervals for hematologic tests for free-ranging northern hairy-nosed wombats (*Lasiorhinus krefftii*).

Hematologic test ^a	Age/sex class	n	Mean \pm SD	Reference interval (5–95%)	Range
Hematocrit (%)	All	35	36.5 \pm 3.0	32–42	30–42
Hemoglobin (g/l)	All	35	122.9 \pm 10.16	110–138	107–149
RBC ($10^{12}/l$)	All	35	4.27 \pm 0.39	3.7–4.8	3.6–5.0
MCV (fl) ^b	Adult males	21	85.52 \pm 3.88	82–90	78–91
	Adult females	10	88.4 \pm 3.06	84–92	83–92
	Subadult males	4	81.25 \pm 2.63	N/A ^c	79–85
MCH (pg/l)	All	35	28.91 \pm 1.33	27.3–30.5	26.0–33.5
MCHC (g/l)	All	35	337.2 \pm 18.96	317–364	314–413
WCC ($10^9/l$)	All	35	7.49 \pm 2.76	3.9–12.2	3.8–13.2
Neutrophils ($10^9/l$)	All	35	5.19 \pm 2.2	3.0–8.9	2.9–11.4
Lymphocytes ($10^9/l$)	All	35	1.57 \pm 1.19	0.2–3.4	0.2–5.0
Monocytes ($10^9/l$) ^d	Males	25	0.36 \pm 0.14	0.1–0.6	0.1–0.6
	Females	10	0.48 \pm 0.13	0.4–0.6	0.3–0.7
Eosinophils ($10^9/l$)	All	35	0.31 \pm 0.28	0–0.7	0.0–1.4
Basophils ($10^9/l$)	All	35	0.06 \pm 0.07	0–0.2	0.0–0.3

^a RBC = red blood cell; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; WCC = white cell count.

^b Differences between the means of males, females, and subadult males are significant ($P < 0.05$). Data are presented separately for males, females, and subadults.

^c N/A = not applicable.

^d Differences between the means of the males and females are significant ($P < 0.05$). Data are presented separately for males and females.

Hematology results

Few significant differences were detected in hematology parameters between males and females, and between subadults and adults. MCV and monocyte numbers were significantly higher in adult female wombats than adult male wombats ($P < 0.05$). MCV was significantly lower in subadult (male) wombats than adult male wombats ($P < 0.05$). No other significant differences in hematologic data were found. To create reference intervals, hematologic data were pooled for both sexes and age classes, except where significant differences between the means occurred (Table 1).

Biochemistry results

No significant differences were detected in biochemistry parameters between males and females. Total protein and globulin values were significantly lower in subadult wombats compared with adult males ($P < 0.05$), and values for urea, ALP, and phosphate were significantly higher for

subadults (males) compared with adult males ($P < 0.05$). To create reference intervals, biochemistry values were pooled for both sexes and age classes, except where significant differences between the means occurred (Table 2).

Microscopic observation of typical northern hairy-nosed wombat blood cell morphology showed both red and white cell morphology to be extremely similar to that described for southern hairy-nosed wombats (*Lasiorhinus latifrons*) (Clark, 2004). Erythrocytes showed mild-to-moderate anisocytosis. Very rare nucleated red cells were identified ($< 1/100$ leukocytes).

DISCUSSION

Limited hematologic data (hemoglobin, hematocrit, and red cell count) have been published for the northern hairy-nosed wombat (Johnson, 1991b). This study presents the first comprehensive collection and analysis of hematologic values and biochemical analytes from this species.

TABLE 2. Mean±SD and reference interval for biochemical analytes for free ranging northern hairy-nosed wombats (*Lasiorhinus krefftii*).

Biochemical parameter ^a	Age class	n	Mean±SD	Reference interval (5–95%)	Range
Glucose (mmol/l)	All	34	7.96±1.66	5.7–10.7	5.4–12
Urea (mmol/l)	Adults	30	7.89±1.52	6.4–9.9	4.9–12.1
	Subadults	4 ^b	10.33±0.8	N/A ^c	9.1–11.2
Creatinine (mmol/l)	All	34	0.3±0.05	0.23–0.36	0.18–0.38
Total protein (g/l)	Adults	30	63.91±6.38	57–73	46–82
	Subadults	4 ^b	57±4.69	N/A	51–62
Albumin (g/l)	All	34	31.97±2.52	27–36	27–38
Globulin (g/l)	Adults	30	32.17±5.66	26–41	17–45
	Subadults	4 ^b	23.25±2.5	N/A	20–26
Total bilirubin (µmol/l)	All	33	2.94±0.79	1.6–4.4	1.5–4.7
ALP (U/l)	Adults	30	149.77±74.7	77–220	72–442
	Subadults	4 ^b	295.75±165.18	N/A	106–468
AST (U/l)	All	33	32.94±12.18	23–57	19–70
ALT (U/l)	All	33	24.82±5.43	18–33	18–42
Creatinine kinase (U/l)	All	33	111.81±148.55	27–213	10–847
Cholesterol (mmol/l)	All	34	3.01±0.58	2.1–3.7	1.7–4.1
Calcium (mmol/l)	All	34	2.39±0.13	2.2–2.6	2.2–2.6
Phosphate (mmol/l)	Adults	30	1.36±0.37	0.9–2.0	0.8–2.4
	Subadults	4 ^b	1.98±0.92	N/A	1.2–3.3
Calcium phosphate ratio	Adults	30	1.87±0.47	1.2–2.4	1.0–3.1
Sodium (mmol/l)	All	34	137.94±4.59	131–144	129–145
Potassium (mmol/l)	All	34	5.07±1.41	3.2–7.1	3.0–9.1
Chloride (mmol/l)	All	34	94.41±6.15	86–103	85–106
Bicarbonate (mmol/l)	All	34	31.35±5.19	25.3–37.8	14.6–39.8

^a ALP = alkaline phosphatase; AST = aspartate amino transferase; ALT = alanine transaminase.
^b Differences between the means of adults and subadults are significant ($P<0.05$). Data are presented separately for adults and subadults.
^c N/A = not applicable.

This study found few statistically significant differences in blood parameters between male and female northern hairy-nosed wombats. Differences must be interpreted with caution due to the low sample size for female wombats. The significantly higher values in females compared with males (numbers of monocytes and MVC) are not readily explained. Although gender-related differences in hematologic parameters have been reported previously in marsupials, no obvious gender-related patterns have emerged (Clark, 2004).

Although statistically significant differences in biochemical values between adults and subadults occurred, these differences should be interpreted with caution due to small sample size for

subadult animals. Elevations in ALP and phosphate in subadult wombats are consistent with findings in young domestic animals, and they are most likely linked to increased activity of the bone isoenzyme ALP associated with bone growth and remodeling (Lorenz and Cornelius, 1993). Lower total protein in subadult wombats, attributable to lower globulins, is also a consistent finding in young domestic animals (Duncan et al., 1994).

The significantly higher value for urea in subadults has several possible explanations. All four male subadults were thin and in poorer condition than most adults. Wombats have a very low maintenance requirement for nitrogen, partly because they have very low rates of renal nitrogen excretion and high rates of urea recycling

through the bacteria of the hindgut (Barboza and Hume, 1992). They also have low rates of protein synthesis (Hume, 1999). Immature wombats would be assumed to have higher rates of protein synthesis, given sufficient nutrient intake, because they would be actively building muscle. An increase in amino acid turnover due to cellular construction may result in increased blood urea levels. However, immature wombats, recently weaned or forced to live independently may be in negative energy balance due to inexperience and lack of milk supplementation. A catabolic state with muscle breakdown may have contributed to increased blood urea levels (Skerratt et al., 1999). In wombats, urea from the liver recycles to the hindgut where it is used by endogenous bacteria. This results in reduced renal loss of urea (Hume, 1999). Immature wombats may have an inadequately developed gut flora, and the incomplete use of urea by bacteria may result in elevated blood urea levels.

The hematologic and biochemical values obtained in this study were comparable with those published for southern hairy-nosed and common (*Vombatus ursinus*) wombats (Gaughwin and Judson, 1980; Booth, 1999; Clark, 2004). Gaughwin and Judson (1980) presented data from 22 free-ranging and eight captive southern hairy-nosed wombats. Samples from free-ranging wombats were collected via cardiac puncture soon after euthanasia. No mention is made of attempts to establish health status or to analyze data for outliers or contamination. These published values have been widely quoted in papers and texts for the past 25 yr. Booth (1999) also provided reference intervals for common wombats. Samples were collected under variable conditions.

Gaughwin and Judson (1980) concluded that southern hairy-nosed and common wombats seem to have greater numbers of WBC than other marsupials, and they speculated that this may be a characteristic of the Vombatidae. This study on northern

hairy-nosed wombats fails to support this speculation. This study indicates the northern hairy-nosed wombat has markedly lower lymphocyte and hence WBC counts than those reported in the other two species.

Gaughwin and Judson (1980) found significantly elevated urea in free-ranging southern hairy-nosed wombats compared with captive animals. They surmised this may have been a result of an adaptation to a low-water environment. Free-ranging wombats were approximately 15% lighter than captive wombats of the same body length, indicating they were in significantly poorer body condition. Drought conditions at the time of that study make it more likely that elevated urea in free-ranging southern hairy-nosed wombats was a result of negative energy balance, as we suggest for subadult male northern hairy-nosed wombats.

The data from this study were used to create the first suggested reference intervals for this critically endangered species. Developing accurate reference intervals in free-ranging wildlife and zoo populations can be challenging. Difficulties include small sample size, nonuniformity of collection and processing methodology, and variation in geographic and environmental factors. In many cases, blood parameters used for reference have not been scrutinized, validated, or analyzed for health status or outliers. This may result in reference intervals that are excessively broad, skewed, or generally inaccurate. In addition, there is potential for large errors when working with data from a small sample size. However, these reference intervals for northern hairy-nosed wombats are expected to be robust and clinically applicable because a large percentage of the known population within a small geographic area was sampled, using uniform capture, sampling, and processing protocols. Data quality was excellent with few outliers, and no contamination or skewing was observed.

There are increasing opportunities for the management and monitoring of the

northern hairy-nosed wombat population. Animals will be translocated to a secondary location, and captive groups will be established. These reference intervals for free-living northern hairy-nosed wombats will provide baseline information to assist in managing the health of this highly endangered species.

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