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BRIEF COMMUNICATION

Clinical chemistry values and tissue enzyme activities in western barred bandicoots *(Perameles bougainville)*

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Key Words

Clinical chemistry, enzymology, marsupial, *Perameles bougainville,* Peramelidae, reference interval

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Background: The western barred bandicoot, *Perameles bougainville,* is an endangered Australian marsupial species whose survival is threatened by a papillomatosis and carcinomatosis syndrome. Investigations to characterize this syndrome would benefit from species-specific clinical chemistry data.

Objectives: The purpose of this study was to determine plasma biochemical reference values and to determine enzyme activities in various tissues of *P. bougainville*.

Methods: Heparinized blood samples were collected by jugular venipuncture from 53 clinically healthy bandicoots of both sexes and at 3 geographic locations. Plasma was analyzed for routine clinical chemistry variables using an automated biochemistry analyzer. Tissues obtained following humane euthanasia of 3 bandicoots were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), α-amylase (AML) and γ-glutamyltransferase (GGT) activities.

Results: Significant differences in results were found for animals based on geographic location and sex, so results were expressed as minimum and maximum values. A population reference interval was calculated for AST activity (20–283 U/L). ALT was found mainly in liver, with lower levels in cardiac and skeletal muscle and kidneys. AST was detectable in many tissues, including heart, liver, kidneys and central nervous system; CK was found in skeletal and cardiac muscle and central nervous system; AML was found in pancreas; and GGT was found mainly in kidneys with less in intestines and pancreas.

Conclusions: These findings will facilitate the interpretation of clinical chemistry results from *P. bougainville* and thereby inform population management and clinical decision-making.

The western barred bandicoot, *Perameles bougainville*, is an endangered Australian marsupial species that once occurred widely across southern mainland Australia until animal and habitat destruction led to its rapid demise.[1–3] Clinical chemistry data from clinically normal *P. bougainville* are lacking, which hinders efforts to monitor the health of natural and captive populations. A papillomatosis and carcinomatosis syndrome has been identified in *P. bougainville* and guidelines for the assessment of clinical chemistry results from affected individuals were required.[4] The purpose of the current study was to develop such reference values for healthy western barred bandicoots from 5 geographical locations in Western Australia. We also measured the distribution of 6 enzymes in 9 tissues.

Three bandicoots were anesthetized using isoflurane gaseous anaesthetic, examined and humanely euthanized due to debilitatation by the papillomatosis and carcinomatosis syndrome (2 females) and an ulcerating scrotum associated with unilateral testicular torsion (1 male). Spleen, lung, liver, heart, kidney, pancreas, small intestine, skeletal muscle, and central nervous system samples were immediately collected from these animals and stored at –20°C for up to 6 months. Each tissue sample was thawed weighed, homogenized and sonicated in phosphate buffered saline according to published methods.[5] The supernatants were analyzed using a RX Daytona automated biochemistry analyzer (Randox Laboratories, Crumlin, UK) for alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1), creatine kinase (CK, EC 2.7.3.2), α-amylase (AML, EC 3.2.1.1) and γ-glutamyltransferase (GGT, EC 2.3.2.2) activities. Enzyme activity was expressed as units (U) per gram of wet tissue weight.

Clinical chemistry data obtained from 53 clinically normal bandicoots were included in the study, including 4 (2 males, 2 females) from Dorre Island; 6 (3 males, 3 females) from

Bernier Island; 23 (15 males, 8 females) from Heirisson Prong; 15 (4 males, 11 females) from Dryandra Woodland; and 5 (4 males, 1 female) from Kanyana Wildlife Rehabilitation Centre. Two samples were from sub-adults at Dryandra and 8 samples were from lactating females at Dorre Island (n = 2), Dryandra (n = 2) and Heirisson Prong (n = 4). Animals were trapped overnight in baited Sheffield or Elliot traps or hand-netted as appropriate, then anesthetized using isoflurane gaseous anesthetic delivered by mask. A thorough clinical examination was performed and blood samples were collected by jugular venipuncture. Approximately 600–800 μL of blood was distributed evenly between two 400-μL lithium–heparin collection tubes (Microtainer, Becton-Dickinson, North Ryde, NSW, Australia). Tubes were centrifuged and the plasma was pipetted into labeled Eppendorf tubes and frozen at -20 °C for up to 5 days prior to submission to the Murdoch University clinical pathology laboratory. Plasma samples were analyzed using a RX Daytona automated biochemistry analyzer (Randox Laboratories, Crumlin, UK) for total bilirubin, calcium, cholesterol, creatinine, glucose, phosphate, urea, total protein, and albumin concentrations, and ALP, ALT, AST and CK activities. Globulins concentration and the albumin/globulin ratio were calculated.

The D'Agostino-Pearson test was used to check data for Gaussian distribution and Levene's test was used to assess homogeneity of variances. The Kruskall-Wallis test was used to compare the means of data sets with homogeneous variances. When raw data sets failed Levene's test, data were transformed using the logarithm, square root or reciprocal of the raw data and transformed data were subjected to the above statistical procedures. Using this protocol, the effects of geographic location, sex and lactation status were investigated for each analyte using SPSS 15.0.0 for Windows (SPSS Inc, Chicago, IL, USA). Statistical significance was set at $P < .05$. A Gaussian tolerance interval was calculated for AST according to published methods.[6]

The activities of ALP, ALT, AST, CK, AML and GGT in various tissues were graphed (Figure 1) and the minimum and maximum values for the 15 clinical chemistry analytes were tabulated (Table 1). Results from bandicoots at different geographical locations were significantly different for ALT ($P = .001$) and CK ($P = .008$) activities, and albumin ($P = .010$), total bilirubin ($P < .001$; n = 49), cholesterol ($P < .001$), glucose ($P = .002$), total protein ($P = .006$), phosphate ($P = .012$), and globulins ($P < .001$) concentrations. Significant differences also were observed between males, females, and lactating females for ALT activity ($P = .002$) and glucose concentration ($P = .015$). Only plasma AST activity had a data set transformable to a parametric distribution, using the reciprocal transformation. The reference interval calculated for AST activity was 20–283 U/L.

To our knowledge, only one previous report has been published of clinical chemistry values for peramelemorphs (bandicoots and bilbies) in which individual values were provided for AST (35, 75 Sigma-Frankel units/L), ALT (76, 43 IU), lactate dehydrogenase (800, 800 Wroblewski units), total protein (4.9, 6.4 g/dL) and albumin (2.75, 3.5 g/dL) for the northern brown bandicoot (*Isoodon macrourus*) and the eastern barred bandicoot (*Perameles gunnii*).[7] As expected, our data from *P. bougainville* were more similar to the results from the congener species *P. gunnii* than to those from the more distantly related species *I. macrourus*.

Significant differences were observed for bandicoots from the 5 geographic locations for the majority of analytes. Genetics is unlikely to explain these differences since all extant *P. bougainville* belong to the same subspecies, *P. bougainville bougainville*[2]. Nutrition, environment, social structures and unavoidable differences in the length of time of sample storage likely accounted for the observed variations.

The tissue enzyme distributions were derived from only 3 animals in poor health and therefore the results may not represent the distribution of enzymes in healthy *P. bougainville*. Preferably, tissue from clinically normal individuals would have been used, but the endangered status of *P. bougainville* precluded this. Nonetheless, the results were consistent among the 3 animals and also similar to findings in healthy dogs.[8]

Increased plasma AML activity is likely to specifically indicate recent or active pancreatic damage. The anatomical proximity of spleen and pancreas in bandicoots, and the occasional presence of pancreatic tissue within the gastrosplenic mesentery, as identified by examination of histologic sections of spleen samples (MDB, unpublished observation), supports the hypothesis that the apparent splenic AML activity likely resulted from the inadvertent inclusion of pancreatic tissue in 1 spleen sample. Based on other tissue enzyme results, elevated plasma CK activity is most likely to indicate recent or active damage to cardiac or skeletal muscle; AST is a non-specific indicator of cellular damage, so results must be interpreted together with the results of other tests and clinical signs.[9] *P. bougainville* had GGT activity in renal, intestinal and pancreatic tissue, but only negligible GGT activity was detected in liver. Based on the high GGT activity in kidneys, urinary GGT activity (relative to urinary creatinine concentration) may be an indicator of renal disease in this species.[9]The ALP activity in liver suggests the potential for induction with hepatobiliary cholestasis.

To our knowledge, this is the first study to determine clinical chemistry and tissue enzymology data from *P. bougainville*. These data will find application in the assessment of clinical chemistry samples from *P. bougainville* for the diagnosis of clinical and subclinical disease and population health monitoring.

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Figure Legend

Figure 1. Enzyme activity in 9 tissue types from western barred bandicoots (*Perameles bougainville*) (n = 3). Each data point is the enzyme activity in U per gram of wet tissue weight.

Table 1. Plasma clinical chemistry results for western barred bandicoots (*Peramales bougainville*) from 5 geographic locations in Australia.*

Analyte	Dorre Island (n = 4)	Bernier Island (n = 6)	Heirisson Prong (n = 23)	Dryandra Woodland (n = 15)	Kanyana Wildlife Centre (n = 5)	*P* Value†
ALP (U/L)	252–534	127–377	122–786	174–754	86–249	.078
ALT (U/L)	46–66	60–115	35–81	45–78	78–202	.001
AST (U/L)*	41–69	33–77	25–224	20–101	26–108	.112
CK (U/L)	173–427	193–840	24–1814	117–368	68–103	.008
Albumin (g/L)	30.3–33.0	31.8–40.0	30.3–38.4	24.4–39.1	34.0–40.8	.010
Bilirubin (mmol/L)	0.7–4.8	1.1–5.6	0–2.5	2.0–6.6	1.1–4.0	<.001
Calcium (mmol/L)	2.07–2.19	1.81–2.20	1.94–2.26	1.87–2.31	2.05–2.25	.276
Cholesterol (mmol/L)	3.3–3.9	3.1–4.0	1.3–7.3	1.9–3.3	2.2–3.5	<.001
Creatinine (mmol/L)	32–41	31–57	29–63	29–43	39–54	-
Glucose (mmol/L)	6.7–8.2	7.0–13.3	5.0–9.5	6.7–10.6	8.0–12.4	.002
Total protein (g/L)	52.3–60.6	53.7–62.1	44.1–57.6	38.6–64.2	52.0–55.9	.006
Phosphate (mmol/L)	1.7–2.2	1.4–2.2	1.1–2.2	1.0–1.8	1.1–1.5	.012
Urea (mmol/L)	11.0–12.0	10.6–17.6	7.8–12.7	6.9–17.8	11.2–13.5	-
Globulins (g/L)	19.3–30.1	21.9–25.6	12.7–20.4	14.0–29.7	12.9–18.7	<.001
Albumin/globulin ratio	1.01–1.71	1.41–1.81	1.82–2.72	1.15–2.34	1.84–3.09	-

*Data are minimum–maximum.

†Kruskall-Wallis test for geographic location using raw or transformed data with homogeneous variances (Levene's test).