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Source: Journal of Wildlife Diseases, 32(1) : 142-146

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.1.142

Journal of Wildlife Diseases, 32(1), 1996, pp. 142–146

Variation in Hematological and Serum Biochemical Values of the Mountain Brushtail Possum, *Trichosurus caninus* Ogilby (Marsupialia: Phalangeridae)

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ABSTRACT: Hematological and serum biochemical values were determined in a wild population of the mountain brushtail possum (*Trichosurus caninus*) at Cambarville, central Victoria, southeastern Australia. Animals were sampled during two-week trapping periods in June, September, and December 1992, and April 1993. Values for hemoglobin, red cell count and hematocrit were significantly higher in males than females. Total protein and mean corpuscular volume (MCV) were significantly higher in female *T. caninus*. Significant seasonal variations were detected for total bilirubin, alkaline phosphatase, total protein, albumin, urea, absolute eosinophils, MCV, sodium, potassium, and phosphate.

Key words: Hematology, serum biochemistry, mountain brushtail possums, *Trichosurus caninus*.

Hematological and serum biochemical analyses may be used to detect organ dysfunction and disease (Whittington and Grant, 1983). This type of information may make it possible to characterize changes in the health of a natural population in terms of the physiological and pathological responses of individual animals (Bradley, 1990). Despite the potential uses of blood chemistry investigations, there have been few attempts to establish reference values for many Australian species (Canfield et al., 1989).

Our objective was to determine the variation in blood chemistry values arising from differences between the sexes, seasons, and female reproductive status of the mountain brushtail possum, *Trichosurus caninus* Ogilby, at Cambarville in central Victoria, southeastern Australia. An additional objective was to establish reference values for the species.

Trichosurus caninus is a herbivorous, nocturnal, arboreal, phalangerid marsupial which inhabits tall wet forests and rainforests in eastern Australia (Owen and Thomson, 1965; How, 1972). Some hematological values in a wild population of *T. caninus* in northeastern New South Wales were reported by Barnett et al. (1979a, b), however serum biochemical values were not determined by these authors.

A population of *T. caninus* was trapped in a 15-ha area at Cambarville (37°34′S, 145°53′E) in central Victoria. The region has been described by Seebeck et al. (1984). The possums were trapped during 2-wk periods in June, September, and December 1992, and April 1993. Animals were sedated by intramuscular injection of Zoletil® (15 mg/kg) (tiletamine hydrochloride and zolazepam hydrochloride in a 1:1 ratio by weight) (Virbac, Sydney, Australia) to facilitate physical examination and the collection of blood samples. In addition, animals were weighed, measured and tattooed for future identification. The pouch of each female was checked to determine reproductive status.

From each animal, 1ml of blood was collected from the jugular vein using a 3ml syringe and 25 g needle and transferred into a blood tube containing ethylene diamine tetraacetic acid (EDTA). Samples were held in a refrigerator at 4 C and then forwarded on ice to Dorevitch Pathology of Camberwell, Victoria. Hematological analyses were completed within 16 hr of blood collection using a Sysmex NE-8000 Automated Hematological Analyser (JOA Medical Electronics Co Ltd., Kobe, Japan). Thin smears were made from the EDTA blood within 1 hour of collection, fixed in methanol and stained with Giem-

TABLE 1. Reference red blood cell values, red cell indices, total white cell and differential cell counts for the mountain brushtail possum, *Trichosurus caninus*, (n = 80) at Cambarville, Victoria, June 1992–April 1993.

Parameter	Median	5th–95th percentiles
Hemoglobin (g/dl)	12.2	10.5–14.1
Packed cell volume (%)	36.0	29.9–42.3
Red cell count ($\times 10^{12}$/l)	4.78	3.99–5.92
Mean corpuscular hemoglobin concentration (g/dl)	34	32–35
Mean corpuscular volume (fl)	74.3	68.1–80.0
Mean corpuscular hemoglobin (pg)	25.5	23.2–26.7
White cell count ($\times 10^{9}$/l)	4.2	2.1–6.8[a]
Neutrophils	2.1[a] (53%)[b]	0.5–4.8[a] (18–79%)[b]
Lymphocytes	1.6[a] (40%)[b]	0.6–3.4[a] (18–75%)[b]
Monocytes	0.1[a] (2%)[b]	0–0.5[a] (0–7%)[b]
Eosinophils	0.1[a] (1%)[b]	0–0.5[a] (0–9%)[b]
Basophils	0[a] (<0.01)[b]	0

[a] Absolute counts: units = $\times 10^{9}$/l.
[b] Percent of total white blood cells.

sa. These stained smears were used to complete differential white cell counts and to examine for the presence of blood parasites.

We collected 1.5 to 2 ml of blood from each animal into a plain serum tube. After clotting had taken place (2 to 3 hr), the blood was centrifuged to separate the serum from the clot. The resulting serum was stored and transported as for EDTA blood. Biochemistry analyses were performed within 16 hr of blood collection by Dorevitch Pathology using a Kodak Ektachem E 700 Automated Biochemistry Analyser (Johnson and Johnson Clinical Diagnostics, Rochester, New York, USA).

Results of hematological and serum biochemical analyses were evaluated for significant differences between the sexes, seasons, and the reproductive status of females. Seasons were defined as winter (June through August), spring (September through November), summer (December through February), and autumn (March through May). As some *T. caninus* were captured more than once over the four seasons, data were unbalanced and included both between and within animal components. Restricted maximum likelihood (Robinson, 1991) was used to estimate random effects for between animal and within animal variability, as well as fixed effects for the factors of interest, including sex, season, and reproductive status. A Wald test (χ^2) was applied to test the significance of the fixed effects (Genstat 5 Committee, 1993). Reference blood values for *T. caninus* at Cambarville were determined by combining all data from all animals, including repeat samples within animals, to determine the median, 5th and 95th percentile values for each parameter.

We captured 33 adult *T. caninus*, 20 females and 13 males, at Cambarville between June 1992 and April 1993. Most animals were trapped at least twice, for a total of 80 captures. Hematological and serum biochemical reference values were established using data from all *T. caninus* captured (Tables 1 and 2). No significant differences between lactating and non-lactating females were found for any blood values.

Significant differences between male and female *T. caninus* were detected for several red blood cell values (Table 3). Hemoglobin, red cell count (RCC) and hematocrit (PCV) were higher in males than females. Mean corpuscular volume and mean corpuscular hemoglobin were greater in females than males. Higher values for hemoglobin, RCC, and PCV in males also

TABLE 2. Reference serum biochemical values for *Trichosurus caninus* (n = 80) at Cambarville, Victoria, June 1992 to April 1993.

Parameter	Median	5th–95th percentiles
Urea (mmol/l)	9.5	5.8–15.8
Creatinine (mmol/l)	0.08	0.05–0.10
Total bilirubin (mmol/l)	7	2–18
Gamma-glutamyltransferase (IU/l)	19	13–39
Alanine aminotransferase (IU/l)	33	13–73
Alkaline phosphatase (IU/l)	1,508	838–2,977
Total protein (g/l)	62	55–647
Albumin (g/l)	38	34–42
Globulin (g/l)	24	19–29
Aspartate aminotransferase (IU/l)	123	79–240
Creatine phosphokinase (IU/l)	418	103–1,676
Lactate dehydrogenase (IU/l)	73	15–627
Glucose (mmol/l)	6.8	5.7–9.0
Amylase (IU/l)	472	242–672
Cholesterol (mmol/l)	2.13	1.52–3.64
Triglycerides (mmol/l)	0.80	0.47–1.61
Sodium (mmol/l)	144	141–148
Potassium (mmol/l)	3.5	2.7–4.9
Chloride (mmol/l)	101	94–108
Bicarbonate (mmol/l)	30	24–34
Calcium (mmol/l)	2.36	2.12–2.53
Phosphate (mmol/l)	1.3	0.8–2.2

have been recorded in *T. caninus* and *T. vulpecula* at Clouds Creek in north-eastern New South Wales (Barnett et al., 1979a) and in *T. vulpecula* in good condition in Victoria (Presidente and Correa, 1981).

No significant differences in the total and differential white cell counts were found between the sexes or the seasons, except for the absolute eosinophil count, which was highest in autumn ($P < 0.001$). Parasitism is a common cause of elevation of eosinophils in the peripheral circulation (Bush, 1991).

Neutrophils were the predominant white blood cell in the peripheral blood of *T. caninus*. This may be a normal feature of this species or it may be the result of a physiological response to handling. Differential and total white cell counts were not performed for *T. caninus* in northeastern New South Wales (Barnett et al., 1979a), hence comparison between the two studies is not possible. Total white cell counts in *T. caninus* were lower than those reported from *T. vulpecula* (Presidente and Correa, 1981). These authors also reported a neutrophilia in conjunction with a relative lymphopenia and eosinopenia in *T. vulpecula* in poor condition, and attributed these changes to stress.

Total protein levels were significantly higher in female *T. caninus* ($P < 0.02$). However, differences in other blood measures could not be attributed to the sex of animals. Seasonal effects were detected for several biochemistry parameters (Table 4). Levels of urea were highest in spring and serum protein levels were highest in summer. Significantly higher serum protein levels in summer also were reported by Barnett et al. (1979a) for *T. caninus* in northeastern New South Wales. Presidente and Correa (1981) did not find significant differences by sex in serum protein levels in *T. vulpecula*; values for urea in

TABLE 3. Variation between sexes in the hematology of *T. caninus* at Cambarville, June 1992 to April 1993. Data were derived from 33 individuals (13 males, 20 females) and include repeat measures for animals captured more than once, giving a total of 80 measures for each parameter.

Parameter	Males mean (SE)	Females mean (SE)	P value
Hemoglobin (g/dl)	12.7 (0.15)	11.9 (0.2)	0.008
Packed cell volume (%)	37.6 (0.7)	34.9 (0.6)	0.006
Red cell count ($\times 10^{12}$/l)	5.1 (0.1)	4.6 (0.1)	0.007
Mean corpuscular volume (fl)	72.2 (0.6)	75.5 (0.5)	0.001
Mean corpuscular hemoglobin (pg)	24.6 (0.2)	25.9 (0.2)	0.001

TABLE 4. Seasonal variation in serum biochemistry values for *T. caninus* at Cambarville (June 1992–April 1993)

Parameter	Winter (n = 18) mean (SE)	Spring (n = 21) mean (SE)	Summer (n = 20) mean (SE)	Autumn (n = 20) mean (SE)	*P* value
Urea (mmol/l)	9.2 (0.6)	12.2 (0.5)	9.3 (0.5)	8.7 (0.5)	0.001
Total bilirubin (mmol/l)	11 (1.5)	12 (1.0)	5 (0.5)	5 (0.1)	0.001
Alkaline phosphatase (IU/l)	1,316 (99)	1,809 (104)	2,135 (107)	1,120 (105)	0.001
Total protein (g/l)	61.8 (0.8)	59.0 (0.1)	62.6 (0.7)	61.2 (0.7)	0.002
Albumin (g/l)	38.7 (0.6)	35.6 (0.5)	37.6 (0.5)	38.4 (0.5)	0.001
Lactate dehydrogenase (IU/l)	481 (84)	187 (27)	96 (14)	85 (25)	0.001
Glucose (mmol/l)	7.0 (0.2)	6.5 (0.2)	6.5 (0.2)	7.1 (0.2)	0.006
Sodium (mmol/l)	147 (0.5)	143 (0.5)	144 (0.5)	145 (0.5)	0.001
Potassium (mmol/l)	4.0 (0.1)	3.5 (0.1)	3.5 (0.1)	3.4 (0.1)	0.001
Phosphate (mmol/l)	1.5 (0.1)	1.6 (0.1)	1.1 (0.1)	1.2 (0.1)	0.001

this species were lower than our findings for *T. caninus*. Elevated dietary protein may be a major factor influencing variation in serum urea levels (Seal et al., 1975; McCue and O'Farrell, 1992). Seebeck et al. (1984) evaluated the diet of *T. caninus* at Cambarville, but these authors did not investigate the nutrient composition of dietary items.

Total bilirubin levels were lower in summer and autumn than winter and spring, while alkaline phosphatase (ALP) was highest in summer. Values for ALP generally were high in *T. caninus* (mean 1631 IU/l; range 777 to 3435) when compared with those from other marsupials (Whittington and Grant, 1983; Canfield et al., 1989). The ALP isozyme in bone may cause an elevation of serum ALP in young growing animals (Seal et al., 1975; Smith and Rongstad, 1980). However, as only adult *T. caninus* were examined in this study, this is unlikely to be a cause of the high levels of ALP that were recorded. These values may be normal for *T. caninus*, as animals appeared to be healthy and there was no concurrent increase in other hepatic enzymes, which may be expected if there was any associated liver pathology (Bush, 1991).

Seasonal variation was detected in serum sodium, potassium and phosphate levels. Serum sodium levels in *T. caninus* were highest in winter. Increased serum sodium levels may result from excessive water loss, decreased water intake, or increased sodium intake (Bush, 1991), which may be related to seasonal dietary variation. Elevation of serum sodium levels in summer in the red kangaroo (*Macropus rufus*) and the euro (*Macropus robustus*) was attributed to variation in the dietary intake of sodium from different species of grass (Dawson and Denny, 1969). Serum potassium levels in *T. caninus* were highest in winter, which may be associated with reduced dietary sodium intake (Bush, 1991). Hence, the seasonal differences in sodium and potassium levels in *T. caninus* at Cambarville may be associated with fluctuating sodium intake due to dietary variation. Studies by Seebeck et al. (1984) and Claridge and Lindenmayer (1993), investigating the diet of *T. caninus* at this site detected significant seasonal variation in the intake of several dietary items, including fungi and the foliage of plants from the forest understorey and ground layers. Phosphate levels were higher in winter and spring than the other seasons ($P < 0.03$). Increased serum phosphates may be attributable to high phosphorus levels in the diet (Bush, 1991).

The serum concentrations of sodium, potassium, and chloride in *T. caninus* were similar to those of *T. vulpecula* in good condition. Both calcium and phosphate levels in *T. caninus* were lower than *T. vul-*

pecula in both good and poor condition (Presidente and Correa, 1981).

The Healesville Sanctuary kindly allowed access to the Veterinary Treatment Centre facilities. Dorevitch Pathology provided prompt, professional processing of the blood samples for hematology and biochemistry. We thank Mr. R. Cunningham and Mrs. C. Donnelly for assistance with the statistical analysis. KLV was supported by an Australian National University Master of Science Scholarship. DBL is a recipient of an Australian Research Council Postdoctoral Award.

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Received for publication 20 February 1995.