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Source: Journal of Wildlife Diseases, 28(3) : 502-506

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-28.3.502

Journal of Wildlife Diseases, 28(3), 1992, pp. 502–506

Effects of Capture on Hematological Values and Plasma Cortisol Levels of Free-range Koalas (*Phascolarctos cinereus*)

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ABSTRACT: Eight free-range koalas (*Phascolarctos cinereus*) were captured on Kangaroo Island (South Australia) and later transported to the Adelaide Zoological Gardens. Blood samples were collected at the time of capture and at 6 hr, 24 hr and 7 days later. Routine hematological analyses were performed and plasma cortisol levels determined. Significant differences were observed between sampling times for erythrocyte numbers, hemoglobin concentrations, packed cell volumes, mean cell volumes, mean cell hemoglobin concentrations, numbers of leukocytes, neutrophils, lymphocytes and lymphocyte:neutrophil ratio ($P < 0.05$). No significant differences were detected between sampling periods in the numbers of monocytes ($P = 0.707$), eosinophils ($P = 0.174$) or plasma cortisol levels ($P = 0.192$).

Key words: Capture, cortisol, hematology, koala, *Phascolarctos cinereus*, stress.

Hematological values are useful values to indicate some diseases in koalas (*Phascolarctos cinereus*) (Canfield et al., 1989a). Since exercise, exertion and "stress" are known to influence hematological values (Obendorf, 1983; Wintrobe, 1976; Swenson, 1977; Ettinger, 1983), the influence of animal handling prior to blood collection should be considered when interpreting hematological tests. This paper describes changes in hematological and blood cortisol values of apparently healthy free-range koalas following capture.

Eight previously unhandled free-ranging adult koalas were captured on Kangaroo Island, South Australia (35°40′S, 137°39′E), under permit by the South Australian National Parks and Wildlife Service, for relocation to the Adelaide Zoological Gardens (34°56′S, 138°36′E). Each koala was captured within 15 min using a noose attached to a pole and restrained in a large hessian bag. Blood samples were collected immediately from the cephalic vein into dipotassium ethylene-diamine tetra-acetate (EDTA) collection tubes. Each animal was carried to a nearby campsite and placed in a transport box. A further blood sample was collected from each koala after 6 hr. The koalas were then transported by ferry and road to the Adelaide Zoological Gardens where further blood samples were collected 24 hr and 7 days after capture. All blood samples were collected with minimal manual restraint.

Hematological analyses were carried out on all samples. Erythrocytes and leukocytes were counted on a Coulter Counter model ZF (Coulter Electronics Pty. Ltd., Brookdale, New South Wales 2100, Australia), hemaglobin concentration was measured by the cyanmethemoglobin method using a Coulter Hemaglobinometer, packed cell volume (PCV) was determined using a microhematocrit centrifuge (HI Clements Pty. Ltd., North Ryde, New South Wales 2113, Australia) and blood smears were stained with Jenner-Giemsa's stain (Dacie and Lewis, 1968). The extent of polychromasia and numbers of Howell-Jolly (H-J) bodies seen in blood smears were recorded as 'occasional' (<1 per oil field) or '+' (1–2 per oil field). Plasma cortisol was measured using a commercially available radioimmunoassay kit (Amerlex Cortisol RIA Kit, code IM.2021, Amersham Australia Pty. Ltd., North Ryde, New South Wales 2113, Australia).

The results were analysed for differences between sampling periods using the STATISTIX computer programme (Analytical Software, St. Paul, Minnesota 55113, USA). The statistical normality of each value was assessed using a Wilk-Shapiro test (Shapiro and Wilk, 1965). Eosinophil and nucleated erythrocyte numbers were not

normally distributed and mathematical transformations (square root, logarithm, arcsin) failed to achieve normality. For these variables, data were grouped to form two-way tables of counts and evaluated for independence between the sampling periods using a chi-square test. In the analysis of lymphocyte:neutrophil (L:N) ratios, one value outside the normal distribution was omitted. Two way analysis of variance techniques were used to assess differences between sampling periods. Observations on individual koalas were not independent since measurements were taken on the same koala over different time periods; to allow for this, the conservative F-test (Greenhouse and Geisser, 1959) was used following analysis of variance to give a conservative P value. Neutrophils, lymphocytes and monocytes were analysed as absolute numbers using total leukocyte counts as a covariate.

Red cell counts/l, hemoglobin concentration, PCV, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), total white cell count, absolute numbers of neutrophils and lymphocytes and L:N ratio differed significantly between sampling periods ($P < 0.05$). Variations in numbers of monocytes, eosinophils, nucleated erythrocytes and cortisol values were not significant.

The highest recordings for red cell count, hemoglobin concentration and PCV were observed at 0 hr and the lowest at 7 days (Table 1). These values decreased between 0 and 6 hr, increased slightly after 24 hr and then declined again to the lowest level at 7 days.

Neutrophil numbers increased more than two fold within 6 hr after capture. Their numbers were lower at 24 hr and 7 days but above those observed at capture. Lymphocyte numbers were lowest at 6 hr then progressively increased. The L:N ratio reflected these changes.

Polychromasia and Howell-Jolly (H-J) bodies were commonly observed in most blood smears. The extent of polychromasia showed a slight decrease after 6 hr but

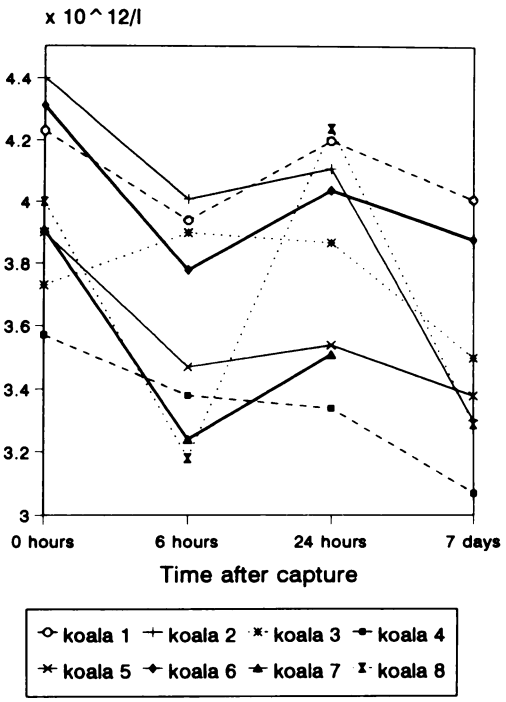

FIGURE 1. Changes in red cell counts over the four sampling periods for individual koalas.

estimates of the numbers of H-J bodies were constant.

Figures 1 to 3 show individual responses at each sampling period for red cell count, PCV and total leukocytes. Most koalas showed a similar pattern to that observed by the group in general. Red cells and PCV were elevated at capture for all except one koala (koala 3), followed by a further increase after 24 hr. Total leukocyte counts were increased at 6 hr except in koala 4. In three koalas (1, 2 and 6) the values for red cells, hemoglobin and PCV at capture were higher than the normal reference range (Canfield et al., 1989b).

The mean levels of blood cortisol were not significantly different. However, cortisol levels in individual koalas appeared to differ (Table 2). Some animals (1, 5, 6 and 8) showed high levels at capture in contrast to others (3 and 4) who showed no detectable response.

A range of stimuli have been described as "stressors" including environmental, physical and psychological factors (Free-

TABLE 1. Mean and standard errors for koala hematological values and cortisol levels for each sampling period.

	Time after capture			
	0 hr	6 hr	24 hr	7 days
Erythrocytes $\times 10^{12}$/l	4.01 ± 0.29	3.61 ± 0.33	3.86 ± 0.35	3.49 ± 0.34
Hemoglobin g/dl	13.8 ± 1.1	12.4 ± 1.2	13.6 ± 1.5	11.5 ± 1.0
PCV l/l	42.9 ± 3.2	38.0 ± 4.2	39.1 ± 3.8	35.1 ± 2.7
MCV fl	107.3 ± 5.6	105.2 ± 5.7	101.5 ± 3.6	100.8 ± 5.6
MCHC g/dl	32.1 ± 1.1	32.6 ± 1.1	34.7 ± 0.9	32.9 ± 0.7
Leukocytes $\times 10^6$/l	5,200 ± 1,248	7,675 ± 2,127	5,187 ± 1,093	8,171 ± 2,723
Neutrophils $\times 10^6$/l (%)	1,757 ± 716 (34)	5,399 ± 1,931 (70)	2,763 ± 499 (53)	3,933 ± 1,227 (49)
Lymphocytes $\times 10^6$/l (%)	3,267 ± 1,268 (63)	2,105 ± 872 (27)	2,335 ± 1,163 (45)	4,105 ± 2,100 (50)
Monocytes $\times 10^6$/l (%)	54 ± 57 (1)	113 ± 121 (2)	83 ± 64 (2)	79 ± 64 (1)
Eosinophils $\times 10^6$/l (%)	122 ± 150 (2)	58 ± 88 (1)	7 ± 19 (0)	55 ± 101 (1)
Nucleated RBC/100 leukocytes	6 ± 4	4 ± 3	5 ± 5	2 ± 1
L:N Ratio	63:34	27:70	45:53	50:49
Cortisol nmol/l	19.8 ± 20.9	7.9 ± 6.5	11.3 ± 12.4	NR[a]
Polychromasia	+	occasional	occasional	occasional
H-J bodies	occasional	occasional	occasional	occasional

[a] NR, not recorded.

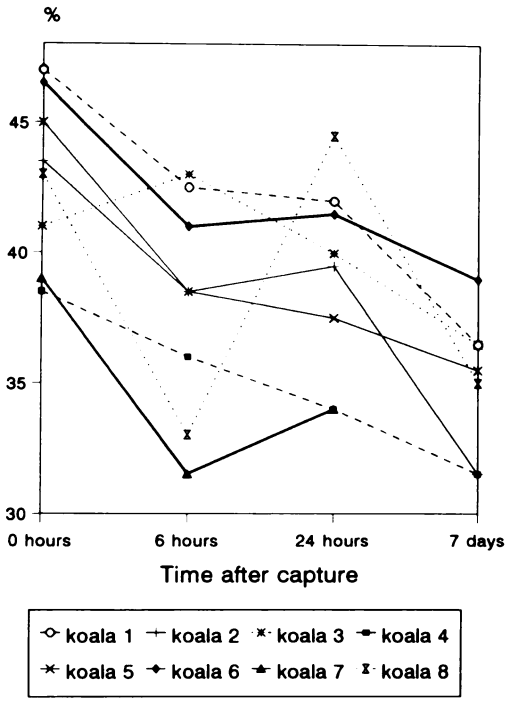

FIGURE 2. Changes in packed cell volume over the four sampling periods for individual koalas.

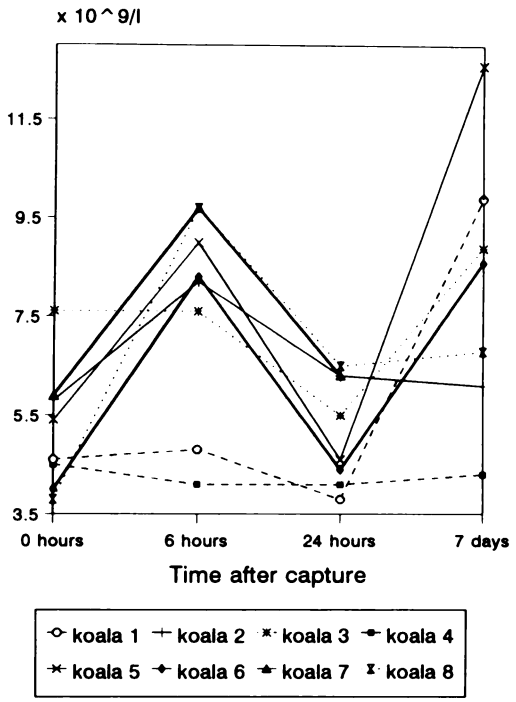

FIGURE 3. Changes in white cell counts over the four sampling periods for individual koalas.

man, 1987). Initial stressors on these koalas at capture were most likely a combination of physical exertion and psychological fear followed later by environmental changes and transport from Kangaroo Island to Adelaide.

In certain species, excitement and strenuous exercise are known to cause contraction of the spleen, expelling stored erythrocytes into the circulation (Jain, 1986; Wintrobe, 1976) resulting in an immediate increase in erythrocyte numbers, hemoglobin, PCV and MCV. Thus, in the koalas, splenic contraction could account for the elevated red cell values observed at capture compared with later sampling periods. Cross et al. (1988) suggests that the release of a larger population of red cells in physically restrained red deer compared to sedated deer may be a result of splenic contraction. This may also explain the high MCV observed at capture in this study.

The changes observed in this study at 6 hours after capture are consistent with a "stress" leukogram, characterised by neutrophilia, lymphopenia, eosinopenia and sometimes monocytosis (Duncan and Prasse, 1986; Jain, 1986), although eosinophil changes were not significant, which may be due to their small numbers. These leukocyte changes are delayed in comparison to immediate erythrocyte alterations at capture, which corresponds with reports of peak neutrophilia and lymphopenia occurring within 4 to 8 hr after corticosteroid stimulation (Duncan and Prasse, 1986; Et-

TABLE 2. Plasma cortisol measurements at each time interval after capture for individual koalas.

Koala	Plasma cortisol (nmol/l)		
	0 hr	6 hr	24 hr
1	51	13	15
2	NR[a]	20	10
3	<5	<5	8
4	<5	<5	<5
5	45	<5	<5
6	25	10	40
7	<5	10	10
8	10	<5	<5

[a] NR, not recorded.

tinger, 1983; Jain, 1986). Reversal of the L:N ratio is frequently considered to be a good indicator of stress or disease in some species (Bollinger and Backhouse, 1960; Dickens, 1975; Obendorf, 1983) and the present study confirms the value of this ratio as an indicator of stress in the koala.

The level of blood cortisol is regarded as an acceptable measurement of stress in some species (Blood and Radostits, 1989). In the present study, differences in cortisol levels were not statistically significant between sampling periods, but this may have been due to the small sample size as reflected in the large standard errors. The high cortisol values at capture followed by a substantial decrease after 6 hr suggests there is some release of cortisol during the distress of capture. Cortisol is present in the plasma in both free and bound forms and, while only the free form is physiologically active, the majority occurs in the bound form (Ganong, 1979). The assay used for plasma cortisol measures total cortisol and an increase in free cortisol may have occurred in response to distress without total cortisol levels having significantly increased.

This study has indicated that capture and handling of free-range koalas has a significant physiological effect on hematological characteristics and that some of these changes may be considered clinically significant. The time of blood sampling in relation to capture is an important consideration when assessing the hematology of koalas.

The authors thank Kevin Mattschoss for his assistance in performing the hematological tests for this study, and J. Arlidge, S. Smith and Ted McKecknie for capture and transport of the animals. The expert statistical advice of Alison Frensham is gratefully acknowledged.

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Received for publication 15 November 1990.