

## ESTABLISHMENT OF SELECTED BASELINE BLOOD CHEMISTRY AND HEMATOLOGIC PARAMETERS IN CAPTIVE AND WILD-CAUGHT AFRICAN WHITE-BACKED VULTURES (*GYPS AFRICANUS*)

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**ABSTRACT:** Despite the devastating collapse of three vulture populations on the Asian subcontinent as a result of their exposure to diclofenac, there is little available information on the normal physiology of many vulture species, including the African White-backed Vulture (*Gyps africanus*). Such information is needed to fully understand mechanisms for toxicity and to identify and prevent future health problems. The aim of this study was to establish baseline parameters for hematologic and selected serum chemistry parameters for this model species for further studies into the toxicity of diclofenac. Captive nonreleasable and wild African White-backed Vultures were used to determine reference values. For hematology, erythrocyte counts, hemoglobin concentration, hematocrit, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, and total and differential leukocyte counts were measured. Chemical analytes measured included sodium, potassium, calcium, albumin, and globulin concentrations, aspartate aminotransferase, creatine kinase, and alanine aminotransferase activities. Uric acid and urea concentrations and the urea:uric acid ratio also were evaluated. Values are presented as means, standard deviations, and reference intervals. The serum chemistry parameters selected may provide a starting point for the evaluation of changes in renal and hepatic function; these organ systems are most severely affected by diclofenac. Results were also compared with values reported for *G. africanus* nestlings, and from these results it is evident that the clinical pathologic parameters are age related. This indicates that the use of nestling values for the evaluation of clinical pathologic findings in adults may be unreliable and could lead to incorrect assumptions.

**Key words:** African White-backed Vultures, clinical pathology, *Gyps africanus*, hematology, serum chemistry, vultures.

### INTRODUCTION

Populations of Oriental White-backed Vultures (*Gyps bengalensis*), Long-billed Vultures (*Gyps indicus*), and Slender-billed Vultures (*Gyps tenuirostris*) on the Indian subcontinent have decreased by more than 98% after exposure to the nonsteroidal anti-inflammatory drug (NSAID) diclofenac over an estimated 15-yr period (Prakash et al., 2003; Green et al., 2004; Oaks et al., 2004). The Oriental White-backed Vulture has been the most affected species. Vultures were exposed to diclofenac while feeding on livestock carcasses that were left to feed vultures. The drug has an estimated lethal dose (LD)<sub>50</sub> in the range of 0.098 to 0.225 mg/kg (Swan et al., 2006), and after exposure, birds die within a few days. Dead birds have lesions consistent with

renal failure, liver injury, and diffuse visceral gout (Oaks et al., 2004; Swan et al., 2006). Swan et al. (2006) reported that African White-backed Vulture (*Gyps africanus*) was as susceptible to diclofenac as the Oriental Black-backed Vulture; therefore, it could be used as an adequate model species to evaluate the toxicity of diclofenac.

Although detailed information is available for blood biochemistry, hematologic and histopathologic changes after NSAID toxicity, relatively little information is available to describe normal values for African White-backed Vultures (Oaks et al., 2004; Shultz et al., 2004; Swan et al., 2006). This knowledge is vital for understanding species' susceptibility to diclofenac and to identify other potential toxic NSAIDs. At present, published hematology and blood biochemistry values for

African White-backed Vulture are restricted to nestlings in South Africa (Van Wyk et al., 1998). Although useful, normal values for nestlings may not be representative for adult birds in which diclofenac toxicity is most likely to occur.

In this study, we report baseline hematologic and blood chemistry values for adult African White-backed Vultures. The parameters selected will allow for a further evaluation of pathophysiology of diclofenac toxicity, because they are indicators of hepatocellular injury and renal function, the two organs (liver and kidney, respectively) most severely affected by diclofenac toxicity.

## MATERIALS AND METHODS

### Collection of blood samples

Wild birds ( $n=25$ ) were caught in Otjiwarango, Namibia, by the Rare and Endangered Species Trust during their routine ringing project in January 2004 and April 2005, respectively. Blood was collected by venipuncture from either the brachial or tarsal vein into sterile syringes with 25-gauge needles. Subsequently, the samples were transferred into evacuated EDTA and serum tubes (Campbell, 1984). Blood samples were centrifuged and serum was stored at  $-30\text{ C}$  until analyzed. With the exception of one subadult, all captured birds were adults. Sex of the birds was unknown.

Captive nonreleasable birds ( $n=21$ ) also were sampled at the DeWildt Cheetah and Wildlife Trust and from the Pretoria Zoological Gardens (PZG). The birds from PZG were nonreleasable due to amputations resulting from previous injuries. The PZG birds were healthy captive breeding stock; 10 were adults and 11 were subadults. Sex of the birds was unknown. All of these birds were deemed healthy based on normal behavior, normal appetite, being of average weight and condition for the species, and a history of being in captivity under daily supervision for at least 2 mo before induction into the study. One week before sample collection, the captive birds were transferred from a communal aviary into individual cages of  $2 \times 2 \times 2\text{ m}$  to minimize the effect of capture and the related corticosterone release on the hematologic profiles. Blood was collected in a similar manner to that used in wild-caught birds. All nonreleasable birds were sampled on three different occa-

sions in August 2004, November 2004, and January 2005.

In addition to the collection of a single sample for baseline determination, two additional nonreleasable birds were subjected to a series of blood collections before and 4, 8, 12, and 24 hr after the feeding of 200 g of fresh meat to allow for interpretation of the urea:uric acid (U:UA) ratio described for the wild birds (Lumeij, 1994).

### Hematology

Thin blood smears were prepared from EDTA samples by means of the slide on slide technique (Campbell, 1984). Smears were subsequently air-dried and immediately stained with Diff-Quik (Kyron Laboratories, Johannesburg, South Africa). The smears were evaluated for cell morphology and the presence of parasites, and a differential leukocyte count was performed. The total leukocyte count (WBC) was evaluated using an improved Neubauer hemocytometer with Turck's fluid (Kerr, 2002).

All other hematologic parameters were analyzed by an automated cell counter (Cell-Dyn 3700, Abbott Laboratories, Abbott Park, Illinois, USA). These were parameters included total erythrocyte counts (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and thrombocyte counts. The Hct was confirmed using the microhematocrit method (packed cell volume [PCV]) described in the literature (Campbell, 1984). The MCV was derived automatically by the cell counter. The Hct was calculated from the following equation:  $\text{Hct} = \text{RBC} \times \text{MCV} / 10$ . The MCHC was calculated as  $\text{Hb} / \text{Hct} \times 100$ .

With the exception of PCV, all hematologic data were derived from nonreleasable birds. Creatinine kinase (CK), albumin (Alb), and alanine aminotransferase (ALT) also were evaluated in the nonreleasable birds to demonstrate normality.

### Serum chemistry

The electrolytes calcium ( $\text{Ca}^{2+}$ ), sodium ( $\text{Na}^+$ ), and potassium ( $\text{K}^+$ ) were measured with a blood gas analyzer (Rapidlab 34E, Chiron Diagnostics, Bayer, Johannesburg, South Africa) using an ion selective electrode method. ALT, aspartate aminotransferase (AST), and CK activities and UA, U, total protein, and Alb concentrations were measured with a Nexet Chemistry Analyzer (Alfa Klasserman, Bayer, Johannesburg, South Africa). Globulin values were calculated as

the difference of the total protein and Alb concentrations. The U:UA ratio was calculated by the following equation:  $U \text{ (millimoles per liter)} \times 1,000 : UA \text{ (micromoles per liter)}$  (Lumeij, 1994).

### Statistical analysis

Statistical analysis was performed with the statistical software SPSS13 (SPSS Inc., Chicago, Illinois, USA). A Kolmogorov-Smirnov and Lilliefors table was used to assess the normality of distribution (Marco et al., 2000). Reference intervals are calculated as arithmetic mean  $\pm 2$  SD for nontransformed data. Where normality was demonstrated using natural log (Ln)-transformed data, the results are presented as geometric mean and SD. Confidence intervals for the Ln-transformed data were back transformed as mean  $(\text{Ln}[X] \pm 2 \text{ SD}(\text{Ln}[X]))$ , where  $X$  represents the clinical pathologic parameter being evaluated (Bland and Altman, 1996). Where normality could not be demonstrated, data are discussed using descriptive statistics. For comparisons, significance was determined using the  $t$ -test.

## RESULTS

The data for normally distributed serum chemistry values from wild birds and hematology values from the nonreleasable birds are presented in Table 1 and Table 2. Parameters for which normality could not be demonstrated are listed in Table 3.

## DISCUSSION

For this discussion, our results for adult vultures are compared with the published values for African White-backed Vultures (van Wyk et al., 1998). Results presented in van Wyk et al. (1998) did not include reference intervals.

The PCV of the wild birds was  $42 \pm 4.06$ , and this value did not differ significantly from that of the nonreleasable birds. The PCV and MCHC values were similar between the captive adult birds and that reported for the nestlings. Both Hb and the RBC values were higher in the adult birds than the 140 g/l and  $1.4 \times 10^{12}$  cells/l

reported, respectively, for the nestlings. The reasons for these age-related differences are not currently understood, but we do not believe that they were related to differences in stress levels due to the handling and sampling of birds. The avian spleen lacks both storage capacity and a muscular capsule, making it physiologically impossible to inject red cells into circulation under stressful conditions (John, 1994; Latimer et al., 2003). It is possible that these differences relate to vulture behavior. Vultures are some of the highest soaring birds, and it is possible that the lower oxygen levels of high altitudes combined with the activity of flight may have contributed to the increased RBC and Hb as a compensatory mechanism in the adult bird (Campbell, 1984; Satheesan et al., 2000). This is supported by Carpenter (1975) who showed that strong fliers in general tended to have a naturally higher RBC. The mean MCV value also was higher in the adult Gyps than for the 161.30 fl reported in nestlings.

The Hct value was also significantly higher than the PCV value in all the birds. Although the exact cause of the difference is unknown, it is likely due to the anticoagulant used. Hematology samples were collected in  $K_3$ EDTA, which is known to induce an artifactual decrease in the PCV while not affecting the Hct (Cott et al., 2003). This decrease may be as high as 2–4% (Abbott Point of Care Inc., 2006).

The mean WBC count was markedly lower than the  $28.59 \times 10^9$  cells/l reported for Gyps nestlings. Unlike other birds (Joseph, 1999; Fudge, 2000; Aengwanich et al., 2002), the segmented heterophil was the predominant leukocyte in circulation. This predominance of heterophils (61%) also has been reported from a Peregrine Falcon (*Falco peregrinus*) nestling (del Pilar et al., 2001). The leukogram also was characterized by the absence of immature (band) heterophils. The high mature heterophil count in the absence of

TABLE 1. Reference hematology intervals for the captive White-backed Vultures ( $n=21$ ).

Parameter <sup>a</sup>	Mean	SD	Min	Max	Reference interval
Hb (g/l)	196.60	16.83	142.00	241.00	162.94–230.25
RBC ( $\times 10^{12}/l$ )	2.55	0.22	2.01	3.03	2.11–2.98
Hct (l/l)	0.50	0.04	0.38	0.60	0.42–0.58
MCV (fl)	196.70	5.55	182.00	211.00	185.60–207.81
MCHC (g/dl)	39.30	1.53	35.40	42.00	36.25–42.35
WBC ( $\times 10^9/l$ )	16.71	1.63	4.00	34.00	13.45–19.97
Het(mat) ( $\times 10^9/l$ )	13.70	6.11	3.04	27.60	1.48–25.93
Het(immat) (band) ( $\times 10^9/l$ )	0.00	0.00	0.00	0.00	0.00–0.00
Lymph ( $\times 10^9/l$ )	1.02	1.91	0.00	12.55	0.00–4.84
Mono ( $\times 10^9/l$ )	1.57	1.04	0.10	5.78	0.00–3.65
Eos ( $\times 10^9/l$ )	0.75	0.71	0.00	3.68	0.00–2.16
Bas ( $\times 10^9/l$ )	0.00	0.00	0.00	0.00	0.00–0.00
PCV (%)	44.30	4.79	31.00	53.00	34.72–53.88

<sup>a</sup> Hb = hemoglobin; RBC = total erythrocyte count; Hct = hematocrit; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; WBC = total leucocyte count; Het(mat) = mature heterophils; Het(immat) = immature heterophils; Lymph = lymphocytes; Mono = monocytes; Eos = eosinophils; Bas = basophils; PCV = packed cell volume.

immature heterophils may be an indicator of a stress or physiologic leukogram. This is supported by Campbell (1984) who suggests that a WBC count higher than  $15 \times 10^9$  cells/l is indicative of stress in tame birds. As such, it is possible that the reported values for the WBC count and heterophils are falsely elevated. No specific data are reported for the Gyps nestlings.

Albumin concentrations were similar to those of Gyps nestlings. The similarity would indicate that serum Alb concentration is finely controlled from an early age and that it is most likely related to the close relationship between Alb and plasma oncotic pressure (Coles, 2005). The mean globulin concentrations were higher in the adults than the 16.6 g/l reported in the nestlings, and this difference is most likely related to increased immune competence resulting from a difference in environmental antigenic stimulation.

Values for sodium and potassium were not normally distributed, and they are presented as 95% confidence intervals. Sodium concentrations were higher than the 130 mM/l reported in Gyps nestlings. The cause of this increase is unknown. Although dehydration may be a possible

cause, the value may be a true reflection for adults, because the concentrations were within the range from 140 mM/l to 160 mM/l reported for three other raptor species (Joseph, 1999; Lierz, 2003). The mean potassium value was higher than the 1.29 mM/l reported in Gyps nestlings but still within the range from 0.6 mM/l to 3.2 mM/l reported in other raptors (Joseph, 1999).

Muscle trauma, which is associated with CK, was minimized by holding birds in individual cages; as such, these results are most likely a fair reflection of the normal values for the species. The results also were within the range from 220 U/l to 500 U/l reported for four other species of raptors (Joseph, 1999). The effect of muscle trauma on CK levels was evident in the wild birds, which struggled endlessly in their attempts of escape from the aviary (Table 3).

Aspartate aminotransferase was measured only in the wild birds, and it was much higher than the mean of 78 U/l reported in Gyps nestlings; this is most likely linked to muscle injury. The ALT and AST values are not specific indicators of liver trauma, because both these enzymes are distributed in other tissues,

TABLE 2. Reference intervals for selected blood chemistry parameters in wild ( $n=14$ ) and captive ( $n=25$ ) African White-backed Vultures.

Parameter <sup>a</sup>	Mean	SD	Min	Max	Reference interval
UA (mM/l) <sup>b</sup>	0.65	0.03	0.22	1.195	0.58–0.724
U (mM/l) <sup>b</sup>	3.21	1.01	1.10	4.70	1.18–5.23
U:UA <sup>b</sup>	5.47	1.39	2.20	12.70	1.39–2.69
Alb (g/l) <sup>c,d</sup>	11.69	1.06	10.50	13.60	10.37–13.18
Globulin (g/l) <sup>d</sup>	21.78	1.14	16	29	16.76–28.30
ALT (U/l) <sup>c,d</sup>	35.51	1.99	6.00	95.00	9.233–144.45
AST (U/l) <sup>d</sup>	1,620.92	2.24	241	5,120	238–6160
CK (U/l) <sup>c,d</sup>	287.47	137.00	136.00	631.00	112.85–596.15

<sup>a</sup> UA = uric acid; U = urea; U:UA = uric acid:urea ratio; Alb = albumin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatinine kinase.

<sup>b</sup> Samples represent arithmetic mean and SD.

<sup>c</sup> Results obtained from the captive birds.

<sup>d</sup> Results represent geometric mean and SD.

especially muscle (Joseph, 1999). As such, we would suggest that increased ALT and AST, in the absence of massively increased CK, would be indicative of hepatocellular injury.

Unlike mammals, U is not a preferred pathway for the excretion of nitrogenous wastes in avians, even though it is fairly well regulated (Lumeij, 1994; Lierz, 2003). Nitrogenous excretion occurs mainly via UA (80–90%) from the proximal convoluted renal tubules. Although a change in either parameter may be an indicator of renal damage or dehydration, this change in function is best evaluated by the ratio of U:UA, in which an increase is indicative of prerenal azotemia (reduced renal arterial pressure, perfusion, or dehydration) and a decrease is indicative of

renal damage. A mean U:UA of 5.45 was present in the adult birds. This value is unfortunately static, and it fails to consider changes in plasma UA brought about by feeding. To allow for an interpretation, the change in the ratio was evaluated in the two additional nonreleasable vultures. After a 24-hr fast, this value was 5.95, whereas after feeding the ratio was 4.74, 1.85, and 2.89 at 5, 12, and 24 hr, respectively. It therefore seems that the value obtained in the wild adult birds represents the unfed state.

The results presented here are an important first step in describing the pathophysiology of diclofenac toxicity in the Asian White-backed Vulture. Although the results for the Gyps nestlings were available as a reference, many of the

TABLE 3. Parameters from wild birds for which normality could not be established.

Parameter <sup>a</sup>	Mean	SD	Min	Max	LCI <sup>b</sup>	UCI <sup>c</sup>
Ca <sup>2+</sup> (mM/l)	1.71	0.77	0.77	2.87	1.49	1.93
K <sup>+</sup> (mM/l)	1.41	0.61	0.66	3.12	1.16	1.67
Na <sup>+</sup> (mM/l)	153.70	12.17	108	176	148.59	158.87
CK (U/l)	16,910	14,988	415	56,080	10,856	22,964
Alb (g/l)	12.79	0.88	11	15	12.41	13.10
TP (g/l)	34.96	3.75	27	43	33.30	36.54

<sup>a</sup> CK = creatinine kinase; Alb = albumin; TP = total proteins.

<sup>b</sup> LCI = 95% lower confidence interval.

<sup>c</sup> UCI = 95% upper confidence interval.



parameters presented were not representative for adult birds.

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