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# Hematological Study of Captive White-Rumped Vultures (*Gyps bengalensis*) to Assess Their Health Status

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**Abstract:** A study was conducted to estimate the hematological values of captive white-rumped vultures (*Gyps bengalensis*) in Nepal. Blood samples were collected from 23 adults and 14 juvenile captive white-rumped vultures during their annual health examinations at the Vulture Conservation and Breeding Center, Kasara, Chitwan, Nepal. Of 23 adults, 12 (52%) were male and 11 (48%) were females, whereas the sex of the 14 juveniles was undifferentiated. The mean ( $\pm$  SD) values for the adult birds were estimated as red blood cell count ( $2.86 \pm 1.01 \times 10^6/\mu\text{L}$ ), white blood cell count ( $14.75 \pm 6.01 \times 10^3/\mu\text{L}$ ), hemoglobin concentration ( $12.86 \pm 1.67$  g/dL), and packed cell volume ( $44.69 \pm 3.63\%$ ). The mean ( $\pm$  SD) values for the juvenile vultures were estimated as red blood cell count ( $1.98 \pm 0.5 \times 10^6/\mu\text{L}$ ), white blood cell count ( $16.73 \pm 7.11 \times 10^3/\mu\text{L}$ ), hemoglobin concentration ( $11.57 \pm 0.39$  g/dL), and packed cell volume ( $44.5 \pm 2.67\%$ ). There were no significant differences between the mean values of the hematological parameters based on the age or sex of vultures.

**Key words:** hematology, conservation, captive, Nepal, avian, white-rumped vultures, *Gyps bengalensis*

## INTRODUCTION

Vultures are natural scavengers and have a significant role in the food chain system by recycling dead and decayed carcasses. Before the 1990s, 9 species of vultures were abundant in South Asia, including Nepal. Among which, the white-rumped vulture (*Gyps bengalensis*) was once considered the most common vulture species in the Indian subcontinent. However, the populations of vultures declined drastically during the past decade of the 20th century. Populations of white-rumped vultures, 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 diclofenac from 1990 to 2005.<sup>1–3</sup> Diclofenac residues in animal carcasses

are toxic to vultures, adversely affecting vulture populations, with the white-rumped vulture being the most adversely affected species.

Different in situ and ex situ conservation approaches were adopted to reverse the population decline and save the last remaining population of vultures in Nepal. One of the ex situ conservation initiatives was started by the establishment of a conservation breeding center in Chitwan National Park (Nepal). With the support of the Nepal government and conservation partners, the Vulture Conservation and Breeding Center (VCBC) was established in 2008 at Kasara, Chitwan, Nepal, in the Chitwan National Park, with the main aim being to ensure long-term survival of globally threatened *Gyps* species of vultures, specifically the white-rumped vulture and the slender-billed vulture. Initially, the white-rumped vulture chicks were captured from their nests when ready to fledge and placed in quarantine for 45 days. Thereafter, the chicks were transferred to the colony aviaries of the VCBC. Sixty white-rumped vulture chicks were collected from Nawalparasi, Rupandehi, Kapilbastu, Dang, Kailali, Kanchan-

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From the National Trust for Nature Conservation, Sauraha, Nepal (Karki, Lamichhane, Sadaula); the Chitwan National Park, Kasara, Nepal (Khadka); and the Bird Conservation of Nepal, Kathmandu, Nepal (Bhusal).

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pur, Arghakhanchi, Palpa, Syangja, and Kaski districts of western Nepal in 2008, 2009, and 2010. Of 60 birds, 57 vultures (95%) were raised in the VCBC, and the remaining 3 vultures (5%) died. The vultures took 5 to 6 years to become sexually mature, and they started breeding activities in 2014. From 5 successive breeding seasons, 16 chicks were successfully hatched from 12 breeding pairs. Among the 16 successfully hatched chicks at VCBC, 1 chick (6%) was attacked and killed by other chicks of the same group after segregation from the parents.

Captive rearing of vultures is a difficult task. Maintaining flock health is a challenge in *ex situ* conservation. Annual health examinations were performed on all vultures to assess their health status. During those annual health examinations, we performed patient assessments, including body weight, heart beat rate, respiration rate, body temperature, and examination of the beak, oral cavity, nares, feathers, pectoral and thigh muscle mass, and feet. In addition to the external physical examination, basic hematological tests are performed including the red blood cell (RBC) count, total white blood cell (WBC) counts, estimation of hemoglobin concentration and packed cell volume (PCV). Basic hematological tests were one of the diagnostic evaluations used to determine the health status of the captive vultures at the VCBC.

Hematological information is useful in helping to determine the general condition and overall health of birds.<sup>4</sup> To rehabilitate individual raptors or to breed and release endangered birds of prey, knowledge of their reference interval concentrations of blood constituents is of paramount importance.<sup>5</sup> Such information can be obtained from blood sampling and simple laboratory techniques.

Relatively few articles have been published on the hematological values of raptor species.<sup>5-13</sup> Data for many parameters, such as the RBC count, WBC count, PCV, hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (MCHC), are scarce for many birds of prey, including different species of vultures. The MCV gives the average volume of individual erythrocytes, which is an indication of erythrocyte size. The MCHC is an expression of the volume within the erythrocyte occupied by Hb, and it reflects the coloration of the RBC. Such parameters are clinically important for diagnosing and monitoring avian medical problems.<sup>4,12</sup> The objective of this study was to provide baseline information on the blood characteristics of different age and sex groups (12 of 23 [52%] were male and 11 [48%] were

female, whereas the sex of the 14 juveniles was unknown) of captive white-rumped vultures.

## MATERIALS AND METHODS

### Collection of blood samples

Approximately 12% (7 of 60) of the total number of white-rumped vultures in captivity older than 8 years were randomly caught during annual health examinations in September of each year. In 2016, 9 of 57 adult vultures (16%) were caught and examined. Likewise, in 2017, 7 out of 60 adult vultures (12%) and another 7 out of 51 adult vultures (14%) in 2018 were caught and examined at the VCBC (Kasara, Chitwan National Park, Nepal). Of these 23 samples, 12 (52%) were male adult vultures and the remaining 11 (48%) were female adult vultures. Clinical examination of the 14 juvenile (8–10 months old) white-rumped vultures, which were hatched at the VCBC breeding center were also performed; 8 chicks (57%) hatched in 2017 were caught in 2018, and 6 chicks (43%) hatched in 2018 were caught in 2019. The sex of these chicks was undifferentiated. With the clinical examination of these vultures, blood was collected by venipuncture from the median tarsometatarsal vein with sterile 3-mL syringes and a 25-gauge needle attached. Subsequently, the samples were transferred into 3-mL ethylenediaminetetraacetic acid (EDTA) tubes (AV Labotube, AV Consumables, Ahmedabad, India).<sup>14</sup> The blood samples were stored in the EDTA tubes at 23°C (73.4°F) until analyzed. All birds sampled were determined to be healthy based on external physical examinations.

### RBC and WBC counts

The RBC and WBC counts were performed following the standard clinical laboratory techniques at the VCBC. For RBC counts, 20  $\mu$ L of the well-mixed EDTA-collected blood sample was mixed well for 5 minutes with 4 mL of physiological saline (0.9% NaCl) in a plastic mixing tube (1:200 dilution). Then, RBCs were counted manually from the  $5 \times 16$  (80 small) squares of one grid of the hemocytometer (an improved Neubauer chamber). The value of RBCs ( $\times 10^6/\mu$ L) was obtained by dividing the total number of cells counted in 5 squares by 100. For the WBC count, 100  $\mu$ L of well-mixed EDTA blood was mixed with 1.9 mL of 1% ammonium oxalate in a plastic mixing tube (1:20 dilution). The WBC was also counted manually from the  $4 \times 16$  (64 large squares) of both grids of the same hemocytometer.

The WBC value ( $\times 10^3/\mu\text{L}$ ) was obtained by dividing the total number of counted cells in 64 large squares by 20.

Estimation of Hb and PCV estimation relied on the colorimetric measurement of Hb released after the lysis of the erythrocytes. The Hb was estimated with a hemometer (Sahli hemometer model 32-430-00, Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany) and measured in grams per deciliter. A combination of 0.2 mL of 0.1-N hydrochloric acid with 20  $\mu\text{L}$  of blood was mixed well for 1 minute; after which, water was added until the brown color matched the glass color comparison bars as closely as possible. The level on the glass tube shows the concentration of the Hb (g/dL). The PCV values were obtained by centrifuging the EDTA anticoagulated blood in a microhematocrit tube at 10 000 rpm for 5 minutes. The centrifuged tube was then placed into a hematocrit reader (REMI Elektrotechnik Ltd, Mumbai, India). The tube was then placed and moved sideways until the top of the fluid column lined up with the buffy coat and the plasma, which allowed the reading of PCV (as a percentage) by following the line to the intersection with the vertical axis of this device.

#### MCV and MCHC

The MCV and MCHC were calculated with standard formulas from RBC, PCV, and Hb concentration.<sup>15</sup>

$$\text{MCV (fL)} = \frac{\text{PCV}(\%) \times 10}{\text{RBC}(\times 10^6/\text{L})} \quad \text{and}$$

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL)} \times 100}{\text{PCV}(\%)}$$

#### Analysis of data

Because there were few samples obtained from each group, the data evaluated for each variable are reported as means, medians, SDs, and minimum and maximum values.<sup>16,17</sup> The mean and variance for each parameter were calculated according to age and sex. The data obtained were entered, and statistical analyses were performed on Excel spreadsheet (Microsoft, Redmond, WA, USA) and RStudio software (version 3.6.1, RStudio, Boston, MA, USA). From the normally distributed data, the mean comparisons with the reference intervals were performed with the Student's *t* test. A two-sample *t* test tool was used to determine the difference between the values of

male and female adults and between adult and juvenile white-rumped vultures. The reference intervals were taken from Naidoo et al<sup>18</sup> and were used to compare the values we obtained.

#### RESULTS

The different blood parameters of the 23 (12 male [52%] and 11 [48%] female) adults and the 14 young white-rumped vultures were studied. No abnormalities were observed in external physical examinations, and the estimated blood values (RBC, WBC, PCV, Hb concentrations, MCV, and MCHC) remained within the reference intervals. The results of the blood tests are listed in Tables 1 and 2.

#### DISCUSSION

The hematological values obtained from the blood samples of VCBC did not differ from the reference intervals obtained from Naidoo et al<sup>18</sup> for both the adult and young white-rumped vultures. The RBC counts of the vultures (both adults and young) at VCBC were not different from the reference intervals ( $P = .07$ ). However, the mean RBC counts for adults were higher than those for the young. An explanation for these age-related RBC differences is not currently known, but it is not thought to be related to differences in stress levels from the handling and sampling of birds.<sup>18</sup> The avian spleen lacks both storage capacity and a muscular capsule, making it physiologically improbable to inject RBCs into circulation under stressful conditions.<sup>19,20</sup> The mean RBC count did not show a sex difference ( $P = .52$ ).

Wild vultures may show increased RBC values because strong fliers, in general, often have higher RBCs.<sup>21</sup> Moreover, to compensate for lower oxygen levels at high altitude, the RBC count and Hb volume are usually increased.<sup>15,22</sup>

The estimated MCV indicates that the average erythrocyte size was within reference intervals for adults ( $P = 1$ ) and for young ( $P = .15$ ), and there was no difference between sexes ( $P = .70$ ). Both male and female adult vultures exhibited statistically similar MCHC values ( $P = .13$ ). The average Hg concentration in RBCs of adult vultures was within the reference interval and can be described as normochromic.

The estimated mean value for the WBCs was found to be within reference intervals. There was no significant difference between the reference intervals and the values obtained ( $P = 1$ ). The WBC count of the young vultures also lay within

**Table 1.** The mean, SD, range, and the *P* value of hematological findings for 23 adult and 14 juvenile captive white-rumped vultures during their annual health checkups at the Vulture Conservation and Breeding Center (Kasara, Chitwan, Nepal).

Parameters	Age	No.	Mean ± SD	Minimum	Maximum	Reference	<i>P</i> value
Hb, g/dL	Adult	16	12.86 ± 1.67	11	17	19.6	.95
	Young	8	11.57 ± 0.39	11	12	19.6	.91
RBC, ×10 <sup>6</sup> /μL	Adult	23	2.86 ± 1.01	1.58	5.15	2.55	.068
	Young	14	1.98 ± 0.5	0.32	2.42	2.55	1
MCV, fL	Adult	16	161.73 ± 48.88	77.67	278.48	196.7	1
	Young	8	350.41 ± 414.36	182.22	1375	196.7	.15
MCHC, g/dL	Adult	16	28.85 ± 3.56	24.16	38.64	39.3	1
	Young	8	26.09 ± 1.74	24.35	28.78	39.3	1
WBC, ×10 <sup>3</sup> /μL	Adult	23	14.75 ± 6.01	5.95	28.5	16.71	1
	Young	14	16.73 ± 7.11	9.65	32.5	16.71	.48
PCV, %	Adult	16	44.69 ± 3.63	40	52	44.3	.37
	Young	8	44.5 ± 2.67	41	49	44.3	.45

Abbreviations: Hb indicates hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; PCV, packed cell volume.

the reference interval, and no significant difference was observed between the reference intervals and the values obtained for the WBC counts of adult vultures. This suggests that the WBC value is unaffected by age in the vultures used for this study. There was also no significant difference between the reference intervals and the values obtained for mean WBCs from the evaluated male and female adult vultures (*P* = .42).

It has been suggested that a WBC count higher than  $15 \times 10^3$  cells/μL is indicative of stress in tame birds.<sup>15</sup> The WBC count for the young vultures exceeded  $15 \times 10^3$  cells/μL. Therefore, this study suggests that capture stress may help explain

higher values or that this value is within reference intervals for younger birds.

The Hb concentration was found to be higher in the adult birds when compared with the juvenile vultures. However, the values did not vary significantly from the reference intervals (*P* > .05). There was also no significant difference in the Hb concentration between male and female vultures (*P* = .28). Similar findings were observed with the PCV reference intervals. No significant difference was observed between the PCV values of adult and young vultures when compared to the reference intervals. Numerically, males had higher PCV values than the female vultures had, but overall the values were not significantly different (*P* = .44).

**Table 2.** The mean, SD, range, and *P* value of hematological findings for 12 male and 11 female adult captive white-rumped vultures during their annual health checkups at the Vulture Conservation and Breeding Center (VCBC) (Kasara, Chitwan, Nepal).

Parameters	Sex	No.	Mean ± SD	Minimum	Maximum	<i>P</i> value
Hb, g/dL	Male	9	12.41 ± 1.04	11	14	.28
	Female	7	13.44 ± 2.19	11.4	17	
RBC, ×10 <sup>6</sup> /μL	Male	12	3.00 ± 1.23	1.78	5.15	.52
	Female	11	2.72 ± 0.90	1.58	4.9	
MCV, fL	Male	9	157.14 ± 43.09	77.67	214.634	.70
	Female	7	167.64 ± 58.52	90.82	278.41	
MCHC, g/dL	Male	9	27.55 ± 2.5	24.17	30	.13
	Female	7	30.53 ± 4.18	26.59	38.63	
WBC, ×10 <sup>3</sup> /μL	Male	12	15.72 ± 7.35	5.95	28.5	.42
	Female	11	13.69 ± 4.2	9.4	25.1	
PCV, %	Male	9	45.28 ± 4.49	40	52	.44
	Female	7	43.92 ± 2.2	41	48	

Abbreviations: Hb indicates hemoglobin concentration; RBC, red blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; PCV, packed cell volume.

Based on hematologic data generated in this study, no significant differences were observed between young and adult white-rumped vultures or between males and females ( $P > .05$ ). The results obtained from this study provide reference intervals that will be useful for evaluating the pathologic conditions and general health of white-rumped vultures. Likewise, comparison of these values with those of wild white-rumped vultures provides an assessment of the performance of these captive birds when released. Vultures, in general, require a higher number of RBCs and Hb concentrations to transport more energy and oxygen for flight at higher altitudes and longer periods of time. A base for reference interval hematological values of captive white-rumped vultures held at the VCBC was determined with this study; however, more research is required to generate a better understanding of the physiologic parameters of these birds.

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