



Research Article

Open Access

**EFFECT OF STRESS ON HAEMATOLOGICAL PARAMETERS OF A JUVENILE OF CRITICALLY ENDANGERED LONG-BILLED VULTURE *GYPSS INDICUS* IN ORCHHA, MADHYA PRADESH, INDIA**

**Sonika Kushwaha**  
B.H.E.L, Jhansi, Uttar Pradesh

Received: August 07, 2015 / Accepted : August 17, 2015  
© Science Research Library

**Abstract**

Vertebrates have evolved specific physiological and behavioural responses to cope with sudden and unpredictable events, such as the attack of predators, psychosocial stressors, or unpredictable weather events. Blood is considered to be sensitive indicator of stress as well as other avian diseases. But since *Gyps* species of vultures are included in Schedule I of Wildlife Protection Act 1972, no attempt was made to collect the blood samples by capturing the vultures. During the study period (2007-2011) only one sick juvenile was found. Blood smears were made in presence of the veterinary doctor and Forest Department. The blood sample was collected in EDTA and blood smears were prepared on the field. There were no significant differences in all hematologic values of the juvenile when compared to the results of established baselines. The PVC and WBC however varied slightly. PVC was 46% that is suggestive of dehydration. WBC was higher ( $25.3 \times 10^9$  cells/L), indicating stress. The ratio of heterophil (1.61) to lymphocytes (4.95) was 0.33, showing optimal degree of stress. The eosinophils of the juvenile were found to be 0.47 109/L. The value of basophils was 0.3 indicating no serious signs of illness. The lymphocyte was 4.95. The value of monocytes in the juvenile was 1.2, thus no chronic diseases. The RBC was normal (1.36), showing the bird was not anemic. Vultures are high 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. The fact that the vultures are free-ranging and lives in Bundelkhand Region means that they are frequently exposed to the adverse effects of heat stress prevailing in the region, particularly during the hot season when the ambient temperature is very high. Keyword:- Water Productivity, Command area, Discharge.

Keywords: Blood, Juvenile, Vultures, Diseases

\*Corresponding author: [sonika2107@gmail.com](mailto:sonika2107@gmail.com)

**Introduction**

Vertebrates have evolved specific physiological and behavioural responses to cope with sudden and unpredictable events, such as the attack of predators, psychosocial stressors, or unpredictable weather events. The values of haematological indices in avifauna can serve as a valuable source of data that result in valuable investigations. The haematological studies are important in assessing the health of the bird as well as provide a way that may lead to correct and timely diagnosis of various pathological conditions. While studying the various aspects of vultures, the investigation of hematological parameters of *Gyps*

Species were included as one of the aims and objectives since blood is considered to be sensitive indicator of stress as well as other avian diseases and it is supposed to be a vehicle for transport of materials within the body. Avian bone marrow contains a large amount of lymphatic tissue as compared to that of mammals. Early studies mostly focused on developing haematological techniques for birds and getting normal values. Later they included studies relating to the effect of disease or certain chemicals affecting the overall health of the birds. Since it is easier to get blood from larger birds, studies tended to focus on large non-passerine birds. The studies were mainly carried out on captive birds in zoos as this was practically easier. Even though haematological parameters are good indicators of the overall condition of the bird (Campbell 1984), very little studies focused on the use of haematological parameters of birds for biomonitoring of environmental conditions. Studies in India concerning pesticides are some of the few examples of haematological studies used in biomonitoring programmes. In veterinary science several haematological parameters are used for the determination of the physical condition of birds. Campbell (1984) discussed these in their practical guide to avian haematology. But since *Gyps* species of vultures are included in Schedule I of Wildlife Protection Act 1972, no attempt was made to collect the blood samples by capturing the vultures. The collection of sample material from wild, adult or juvenile vultures of any species is a task that poses a vast array of problems and difficulties. During the study period (2007-2011) only one sick juvenile of *Gyps indicus* was found in Cenotaphs of Orchha in Tikamgarh District of Madhya Pradesh (fig 1).



Fig 1: Sick juvenile of *Gyps indicus* was found in Cenotaphs of Orchha

**Study Area**

Tikamgarh is one of the northern districts of Madhya Pradesh and it is located between north latitude 24°26' to 25°34' and east longitude 78°26' to 25°34'. The total geographical area of Tikamgarh District is 5048.00 Sq. Km. Orchha lies in Tikamgarh

district of northern Madhya Pradesh. It lies between the Jamni, a tributary of Betwa and Dhasan rivers (fig. 2). The year may be divided into four seasons. May is generally the hottest month with mean daily maximum temperature at about 43 degree Celsius and low 29 degree Celsius. On individual day temperature may rise upto about 47 degree Celsius. The driest part of the year is summer season when the relative humidity is less than 20 percent in the afternoons.

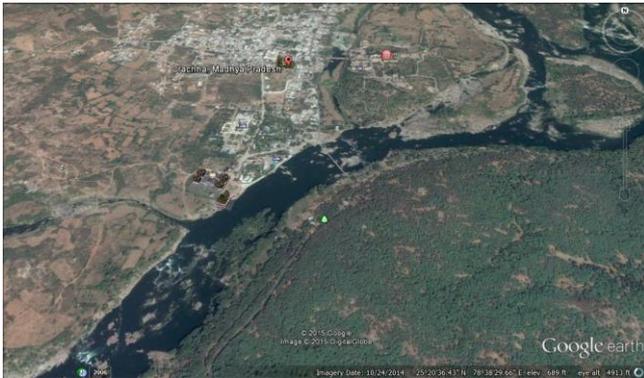


Fig 2: Map of Orchha, Tikamgarh (Source: Google earth)

### Methodology

Blood smears were made in presence of the veterinary doctor and forest Department. However only limited parameters could be studied as the sight of sample collection was far from the laboratory. The blood was collected from the wing vein using a disposable 23-gauge needle and 2-ml plastic syringe containing EDTA as anti-coagulant. The blood was used to prepare blood smears. Fix the air-dried film in methanol for two minutes. The smear was allowed to dry and then immersed in a solution of 1 part of Giemsa stock solution to 10 parts of buffered water (pH 7.2) for 45 min. The slides were discarded thoroughly in vertical position and allowed to air dry. The bottom side of the slide was wiped before drying. The stained smears were mounted in a neutral mounting medium (Canada balsam or DPX).

### Results and discussion

Since the hematological study was not undertaken to establish a baseline of hematological parameters, the result (Table 1) was evaluated with the help of hematological baseline established by Naidu, Diekmann, Wolters and Swan in 2008 and by M. Hernandez and A. Margalida in 2010. There were no hematozoa detected in the blood samples. No Trypanosomes were reported although they were reported by Wenyon (1926) in vultures. Nothing however is known regarding their pathogenicity. Although the blood protozoa *Haemoproteus* species and *Leucocytozoon* species were not reported in this study, there are however few records in the literature. The juvenile of *Gyps indicus* did not show any clinical symptoms of anorexia, emaciation, diarrhoea, lessen consumption of water or ruffled feathers that are usually associated with birds infected with capillaria. The haematological parameters, such as Packed Cell Volume (PCV), Haemoglobin (Hb) and Total Protein (TP) are significant in evaluating the health condition and disease of birds (Hawkey *et al.*, 1983; Hawkey *et al.*, 1984).

There were no significant differences in all hematologic values of the Juvenile when compared to the results of established baselines. The PVC and WBC however varied slightly. PVC was 46% while PVC more than 42%, in large birds and more than 55% in small birds is suggestive of dehydration or polycythemia (Terry W. Campbell). Polycythemia is rarely in birds (Taylor, 1987). The value of PCV is an excellent indicator of the haemogram, and principally of the number of circulating erythrocytes and Hb (Benjamin, 1985).

Table 1: Hematological parameters of the sick and dehydrated Juvenile Vulture (VJ).

S. No.	Parameter	Value
1	Hb (g/L)	138
2	RBC ( $\times 10^{12}/L$ )	1.36
3	MCV(fT)	167.83
4	MCHC(g/dL)	25.9
5	WBC ( $\times 10^9/L$ )	25.3
6	Lymph ( $\times 10^9/L$ )	4.95
7	Heterophils ( $\times 10^9/L$ )	1.61
7	Mono ( $\times 10^9/L$ )	1.2
8	Eos ( $\times 10^9/L$ )	0.47
9	Baso ( $\times 10^9/L$ )	0.3
10	PCV(%)	46

The body's immune system involves the White blood cells (WBC's) as a defense. There are five types of WBC's found in birds. Heterophils (hets), eosinophils (eos) and basophils (baso), lymphocytes (lymph) and monocytes (mono). The first three are known as granulocytes due to the occurrence of colored granules in their cytoplasm. Leukocytosis is the increase in the WBC count. Leukocytosis may commonly occur due to the genuine transporting and handling of a bird at the treatment centre. This is a physiological alteration owing to the stress come upon the bird. The normal percentage of heterophils is between 40-75%. It is changes in numbers and characteristics can occur which considered the "window to the bird's state of health" since even subtle problems such as trauma, minute infection, and mild irritation. The WBC was also higher i.e.  $25.3 \times 10^9$  cells/L which indicate stress or physiological leukogram. This is supported by Campbell (1984) who suggested that a WBC higher than  $15 \times 10^9$  cells/L is indicative of stress in tame birds.

The ratio of heterophil (1.61) to lymphocytes (4.95) was 0.33, showing optimal degree of stress. The heterophil to lymphocyte ratio is now generally established as a consistent and accurate physiological indicator of the stress response in chickens (Gross and Siegel, 1983), because exposure to stressors causes it to increase progressively. Gross and Siegel (1993) suggested that reference values for the heterophil to lymphocyte fraction of about 0.2, 0.5 and 0.8 attribute to little, optimal and elevated degrees of stress, correspondingly.

The number of eosinophils occurs in very small numbers as compared with the normal range i.e 0-2%. The eosinophils of the juvenile were found to be 0.47  $10^9/L$ . Function of the avian eosinophil is unclear; however, enhanced numbers are typically associated with tissue damage, parasitic infections, or may be allergic reactions. Basophils (baso) contain cytoplasmic granules that are dark-stained (basophilic) and thus identified easily. They also occur in small numbers with a normal range of 0-5%. The value of basophils in the juvenile was 0.3. The exact function of the basophil is unknown. Elevated numbers are frequently connected with persistent and extended poor health. They also appear to play a role in the early stages of inflammation. Here the value was normal, showing no serious signs of illness. Lymphocytes may increase in numbers (lymphocytosis) in lymphoid leukemia and certain types of chronic infections. They reduce in figures in heightened viral infections. The lymphocyte in case of the juvenile was 4.95. Monocytes are the largest WBC's found in avian blood and they are very similar in appearance to lymphocytes. They are present in minute numbers with a standard range of 0-3%. An increase in the percentage of monocytes (up to 10%) is seen with certain chronic diseases such as fungal infectivity, tuberculosis, and enormous tissue damage. The value of monocytes in the juvenile was 1.2, thus no chronic diseases. Examination of the erythrocytes is related with the possibilities of anemia-related problems and improper functioning of the bone

marrow response (which is where RBC's are produced). In the Juvenile vulture, most RBC's were mature with a small percentage of immature erythrocytes pointing towards a normal replenishment from the bone marrow. Immature RBC's were slightly larger than mature RBC's. As a bird becomes anemic, the number of RBC's decreases. The RBC is normal here i.e. 1.36, showing the bird was not anemic. 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). These differences may be related to vulture behavior. Vultures are high 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.

### Conclusion

The fact that the vultures are free-ranging and survive in Bundelkhand Region means that they are frequently exposed to the adverse effects of heat stress prevailing in the region, particularly during the hot season when the ambient temperature is very high. The results represent an important first step in providing effect of stress on haematological parameters of a juvenile of Critically Endangered vulture *Gyps indicus* in Orchha, India. The reference values obtained are useful and will contribute to the previous data that are available for various vulture species. Further studies are recommended to study the avian hematologic and blood chemistry responses to disease that may serve as a tool in the assessment of pathologic conditions of the Critically Endangered species.

### Acknowledgment

I am grateful to Prof.M.Tripathi, Head, Department of Zoology, University of Lucknow for providing necessary facilities during the research work. Thanks are due to the Archaeological and Forest Department of Madhya Pradesh for providing the support as well as necessary permission to carry out the study. I am also thankful to Prof.A.Kanaujia, Department of Zoology, University of Lucknow who provided the guidance. Thanks are extended to Dr. A.K.Chhangani, M.G.S University, Bikaner for constant encouragement.

### References

- Benjamin MM (1985). Outline of veterinary clinical pathology. 3rd ed. Kalyani, Publishers, New Delhi, India.
- Campbell T W (1984). Avian hematology. The basic. Veterinary Clinics of North America. Small Animal Practice 14:223-248.
- Carpenter FL (1975). Bird Hematocrits: Effects of high altitude and strength of flight. Comparative Biochemistry and Physiology 50A:415-417.
- Google earth
- Gross WB and Siegel HS (1983). Evaluation of the heterophil to lymphocyte ratio as a measure of stress in chickens. Avian. Dis. 27:972-979.
- Gross WB and Siegel PB (1993). General principles of stress and welfare. Pages 21-34 in: Livestock, Handling and Transport (T. Grandin, ed.). CAB International, Wallingford, UK
- Hawkey C, Hart MG, Samour HJ, Knight JA and Hutton RE (1984). Haematological findings in healthy and sick captive Rosy flamingos (*Phoenicoptus ruber ruber*). Avian Pathol. 13:163-172.

- Hawkey C, Samour JH, Ashton DG, Hart MG, Cindery BN, Finch JM, Jones DM (1983). Normal and clinical haematology of captive cranes (Gruiforms). Avian Pathol. 12:73-84.
- Hernandez M and Margalida A (2010). Hematology and blood chemistry reference values and age-related changes in wild Bearded vultures (*Gypaetus barbatus*). Journal of Wildlife Diseases 46(2):390-400
- John J. L. (1994). The avian spleen: A neglected organ. Quarterly Review of Biology 69:327-351.
- Latimer KS, Mahaffey EA, Prasse KW and Duncan JR (2003). Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. Iowa State University Press, Ames, Iowa, pp. 46-80.
- Naidu V, Diekmann M, Wolters K and Swan GE (2008). Establishment of selected baseline blood chemistry and hematologic parameters in captive and wild-caught African White-backed vultures (*Gyps africanus*). Journal of Wildlife Diseases 44(3): 649-654.
- Satheesan SM (2000). Vultures in Asia. In: Raptors at risk, eds.
- Taylor M (1987). Polycythemia in the blue and gold macaw: A case report of three cases. Proc 1<sup>st</sup> Intl Conf Zool & Avian Med, pp. 95-104.
- Wenyon CM (1926). Protozoology. A Manual for Medical Men, Veterinarians and Zoologists. Vols. I and II Bailliere, Tindall and Cox, London.



Science Research Library (SRL) Open Access Policy

SRL publishes all its journals in full open access policy, enables to access all published articles visible and accessible to scientific community.

SRL publishes all its articles under Creative Commons Attribution - Non-Commercial 4.0 International License



Authors/contributors are responsible for originality, contents, correct references, and ethical issues.

Author benefits:

- ✓ Online automated paper status
- ✓ Quality and high standards of peer review
- ✓ Rapid publication
- ✓ Open Access Journal Database for high visibility and promotion of your research work
- ✓ Inclusion in all major bibliographic databases
- ✓ Access articles for free of charge