



Haematological, Biochemical and Cytomorphometric analysis of an Indian Pangolin

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Abstract

Haematological and serum biochemical values of a sick, adult female Indian pangolin (*Manis crassicaudata*) were determined. Results obtained showed a higher total leukocyte count, low haemoglobin value and erythrocyte count in comparison to haematological values reported on other pangolin species. The study also determined cytomorphometric values for erythrocytes and leukocytes. The present paper also described red cell polymorphism in Indian pangolin.

Keywords: Pangolin, *Manis crassicaudata*, gastroenteritis, restrain procedure, RBC polymorphism.

Introduction

Pangolins are native to Asia and Africa and represented by eight extant species belonging to the family Manidae of order Pholidota. Indian pangolins (*Manis crassicaudata* Gray, 1827) are distributed throughout peninsular India, Sri Lanka, Bangladesh and Pakistan¹⁻³. Hunting for local consumption and international trade of their skin, scales and meat; loss and deterioration of habitat are major threats to pangolins^{3,4}. The Indian Pangolin is included under Appendix II of CITES, as 'Endangered' under the IUCN Red List³, and as a Schedule I animal in the Wildlife (Protection) Act, 1972.

Assessment of blood parameters in animals may guide the evaluation of physiological, nutritional and pathological conditions of animals⁵. Literature on haematology, serum biochemistry and cytomorphometric parameters of blood cells of pangolins are sparse. The reason behind the lack of information is their nocturnal and fossorial mode of life, low survival rate in captivity. It is difficult to collect blood from pangolins, as their entire body is covered with scales (except snout, ventrum and foot pad) and they become coiled on defence making their body parts inaccessible for blood collection. Information on haematological, of pangolins is scanty. Haematological and biochemical values were reported in the captive Chinese pangolin (*Manis pentadactyla*) and white bellied pangolin (*Manis tricuspis*)⁶⁻⁸. Haematological values as well as blood chemistry for the Indian pangolins could not be found from available literature. Therefore, there is a need to document haematological parameters and compare them with information available for other pangolin species that have already been reported. The present paper is intended to evaluate blood samples collected from a sick adult female Indian pangolin in order to provide information about some haematological, biochemical and cytomorphometric values in a

physiologically altered condition, which can be used as a reference to carry out further research.

Material and Methods

Animal housing and husbandry: Nandankanan Zoological Park, Bhubaneswar, Odisha received an adult female Indian pangolin (*Manis crassicaudata*) from Rourkela, Odisha on 28.06.2013. The pangolin weighed 8.2 kg and measured 97cm from tip to tip with a 47cm long tail. The newly received pangolin was kept in quarantine for 30 days. About 600g of red weaver ants (*Oecophylla smaragdina*) was provided as feed. During quarantine period deworming of the pangolin was carried out with 6ml of Albendazole. After completion of quarantine period, the pangolin was shifted to an enclosure of 4.2X4.8X3m dimension with provisions for food, water and hollow wooden log as enrichment material.

Blood and mucus was observed in the faeces on 21.08.2013. The animal became off-fed and exhibited tail dragging (a sign of weakness or sickness). While handling the pangolin for treatment, blood sample was collected. Despite of treatment by zoo veterinary wing i.e., intravenous administration of broad spectrum antibiotics (Amikacin, 10mg/kg body weight) and vitamin-K, the pangolin died in the same day. Post-mortem findings revealed that the death was due to gastroenteritis associated with hepatitis and nephritis.

Blood sample collection: Previous studies have described physical and chemical restrain procedures for collection of blood sample from pangolin^{6,9,10}. Historically blood samples of pangolins were collected by toe clipping⁶ and cardiac puncture^{7,9}. But blood sampling via caudal venipuncture was an established method used for haematological studies and routine veterinary procedures^{6,10}. Blood sampling from pangolins using

a 23-G $1\frac{1}{4}$ inch needle was reported¹⁰. In the present study blood samples were collected from a sick adult female pangolin on 21.08.2013 at Pangolin Conservation Breeding Centre, Nandankanan Zoological Park, Odisha, India. As the animal was sick and easily become uncoiled, 7ml of blood was collected by caudal venipuncture using 23-G $1\frac{1}{4}$ inch needle by mild physical restrain of the animal. Then four ml of blood was collected into sampling vials containing Ethylene-diamine-tetraacetic acid (EDTA), from which one ml of blood was used to prepare smears for cytological studies and remaining three ml of blood was used for other haematological studies, viz., determination of haemoglobin concentration, total leukocyte count etc. Another three ml of blood was collected without any chemical and left to clot. Serum was collected from it by centrifugation of supernatant at 2,000 rpm for 15 minutes and stored in subzero temperature till analysis. The samples collected were immediately taken to the laboratory for analysis.

Haematology and serum biochemistry: Total leukocyte count and erythrocyte count were determined using a haemocytometer. Haemoglobin concentration was determined by indirect acid haematin method^{11,12}. The microhematocrit tube, utilized for manual packed cell volume (PCV) determination, was centrifuged for 5 min at 5,000 rpm and interpreted by visual inspection against a standard calibration using the standard method¹³. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined as per Weiss and Wardrop¹⁴. Spectrophotometer based serum biochemical tests (total protein, glucose, cholesterol, urea, creatinine) were carried out using standard kits (Crest biosystems, Alto Santacruz Bambolim complex, Goa-403 202, India). Slides (25.4X76.4X1mm dimension; Riviera™) with blood smears were air-dried and stained with Leishman's stain (RANKEM, RFCL, Ltd., New Delhi, India) for 10 minutes. After air drying, the smears were observed under microscope (Microscope H 600 Wilozyt plan, Helmut Hund GmbH, Wetzlar-Nauborn, Germany) for differential leukocyte count. Same slides were used for cytomorphometric evaluation of blood cells i.e., Red blood corpuscles (RBC) and White blood corpuscles (WBC). Measurements of the blood cells and microphotographs were taken with 400X magnification using Microscope Eyepiece Digital Camera (CatCam130 – 1.3 Megapixel, Catalyst Biotech, Maharashtra, India) attached to the microscope. The present study employed standard procedures for classification of erythrocyte polymorphism described elsewhere^{15,16}. Brief, following terms are used to describe erythrocyte polymorphism, i. anisocytosis- variation in RBC size, ii. poikilocytosis- variation in RBC shape, iii. dacryocytes-teardrop shaped RBCs, iv. drepanocytes- sickle shaped RBCs, v. spherocytosis- spiculated RBCs with spherical shape and evenly dispersed short projections, vi. elliptocytosis- spiculated RBCs oval shape and evenly dispersed short projections, vii. elliptocytes-oval shaped RBCs, and viii. keratocytes-RBCs with 1-2 spicules.

Results and Discussion

Haematology and serum chemistry values are among the most commonly used indices in the clinical evaluation of diseases, both for animals maintained in controlled environment and for free ranging animals¹⁷. The present study reports the haematological and serum biochemical values of a sick adult female Indian pangolin (table 1). The Indian pangolin under study, blood collection procedure and different leukocytes observed were presented in the figure 1.

Table-1
Some haematological and serum biochemical values of the sick female Indian pangolin

Parameters	Values for females Indian pangolin
PCV (%)	20.2
RBC ($10^6/\text{mm}^3$)	2.8
HB (g/dl)	7.5
MCV (fl)	72.14
MCH (pg)	26.78
MCHC (%)	37.12
WBC ($1,000/\text{mm}^3$)	25.9
Lymphocytes (%)	59
Neutrophils (%)	18
Monocytes (%)	12
Eosinophils (%)	11
Total Protein (g/dl)	7.17
Urea (mg/dl)	87.56
Creatinine (mg/dl)	0.25
Cholesterol(mg/dl)	113.71

Since baseline value for haematology and serum chemistry value are not available for Indian pangolin in the available literature, the results of the study were compared with the base line values of other pangolin species. Haemoglobin concentration (7.5g/dl) of the sick Indian pangolin was found low in comparison to previous studies on other pangolin species, i.e., 13.68 ± 1.38 g/dl in female Chinese pangolins⁶ and male 9.82 ± 1.6 gm/dl and female 10.2 ± 1.4 g/dl white-bellied pangolins⁷.

The present study revealed an increased value for total leukocyte count ($25,900/\text{mm}^3$) in the sick Indian pangolin, in comparison to that of female Chinese pangolins reported by Heath⁶ ($4,800-10,700/\text{mm}^3$). Oyewale *et al.*⁷ reported total leukocyte count in male ($5.3 \pm 2.45 \times 10^9/l$) and female ($4.3 \pm 1.79 \times 10^9/l$) white-bellied pangolins. Differential leukocyte count revealed lymphocytes (59%) as dominant white blood cell type followed by neutrophils (18%), monocytes (12%) and eosinophils (11%). Basophils could not be observed during the study. Total leukocyte count varies with species and is influenced by age, stress-induced corticosteroid or epinephrine release due to anaesthesia, capture, handling, and transport, as well as disease and allergic reactions^{5,18}. In most

species for which reference ranges have been established, the neutrophil/heterophil count is a useful indicator of infection¹⁹.

Lymphocytes may increase in chronic infections, whereas neutrophils may increase during acute infections¹⁸.

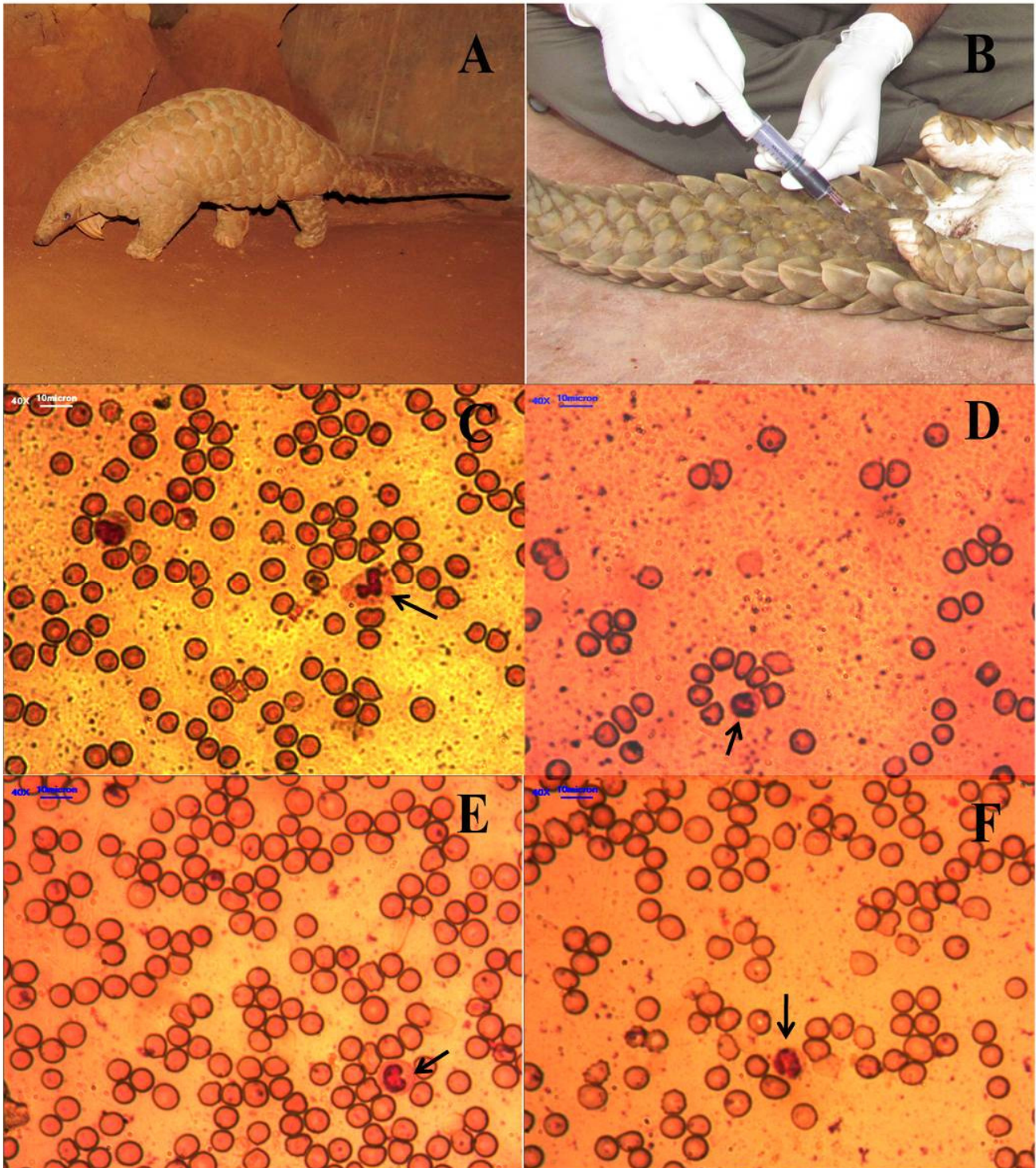


Figure-1

A) Rescued female Indian pangolin (*Manis crassicaudata*) housed in Pangolin Conservation Breeding Center, B) Blood collection procedure, C) Neutrophil, D) lymphocyte, E) Monocyte, F) Eosinophil of the female pangolin

Erythrocytes are approximately 8 μm in diameter. Besides normal red blood cells (normocytes) which accounts for 59%, examination of blood smears revealed the presence of abnormal red blood cells such as dacryocytes (6%), drepanocytes (5%), echinocytes (11%), elliptoechinocytes (9%), elliptocytes (7%) and keratocytes (3%) (figure-2). These blood cells were measured and tabulated (table-2). Any consistent increase in poikilocytosis and anisocytosis was believed to be associated with change in health status¹⁷.

The data presented in this study, may serve as an important reference point for future health-related and diagnostically integrated health studies of Indian pangolins. Cytological parameters obtained in this study can be useful in detecting and monitoring health status of pangolins in general and Indian pangolin in particular.

Table-2
Cytomorphometric values of blood cells of a diseased female Indian pangolin

Cytomorphometry	Length(μm)	Breadth(μm)
Lymphocyte (N=20)	9.88±1.13	9.43±1.64
Neutrophil (N=20)	14.86±3.55	12.97±3.21
Monocyte (N=20)	10.67±1.2	9.04±1.83
Eosinophil (N=20)	13.16±2.51	10.76±3.08
RBC (normocytes) (N=20)	8.09±0.55	7.95±0.62
Dacryocytes (N=20)	9.66±1.02	6.62±0.72
Elliptocytes(N=20)	9.48±1.78	5.67±0.61
Drepanocytes (N=10)	*	4.39±0.47
Spheroechinocytes (N=20)	7.14±0.39	6.9±0.45
Elliptoechinocytes (N=20)	8.46±1.18	5.51±0.64
Keratocytes (N=10)	8.09±1.28	6.31±0.83

*Length could not be measured due to its sickle shape

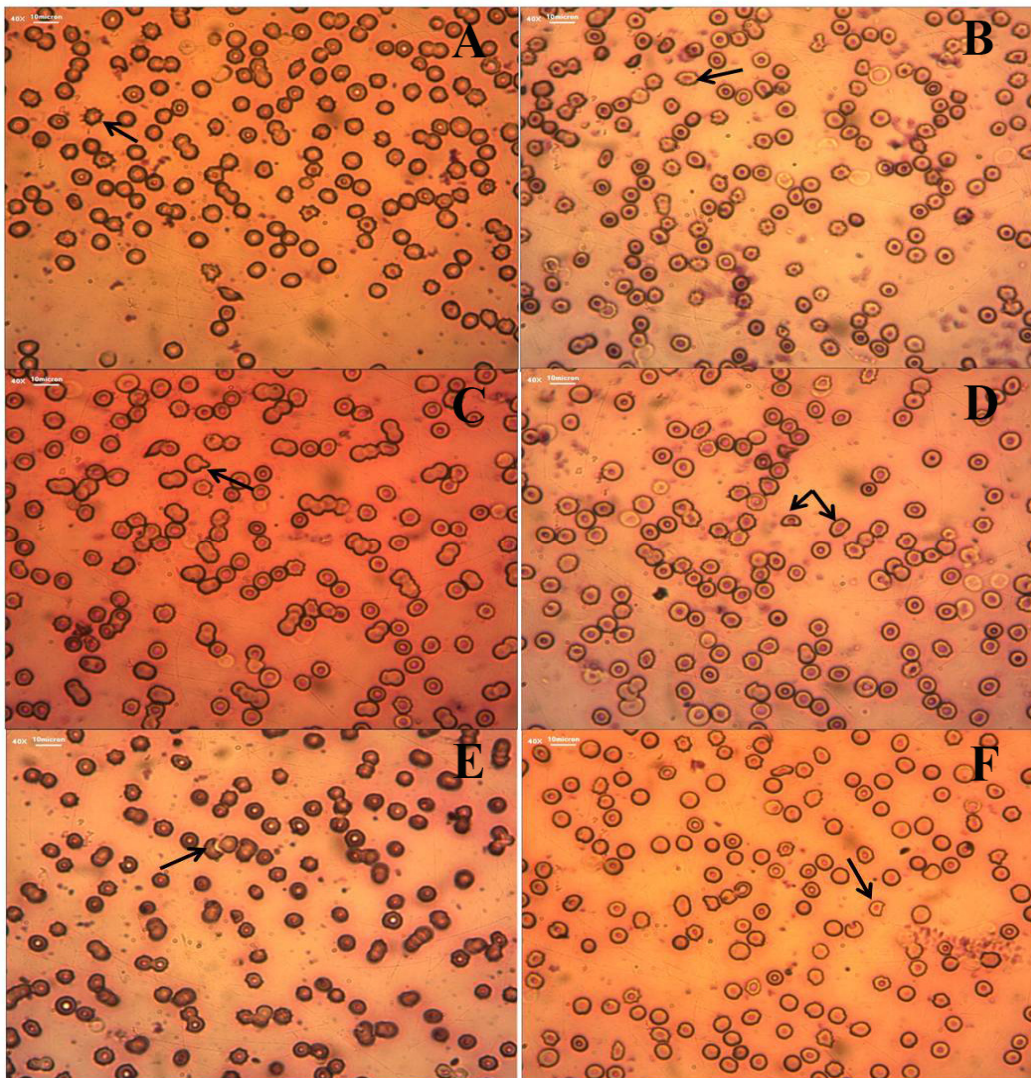


Figure-2

Red cell polymorphism in Indian pangolin, A) Spheroechinocyte, B) Elliptoechinocyte, C) Dacryocyte, D) Elliptocyte, E) Drepanocyte, F) Keratocyte.

Conclusion

This study has presented information comprising haematological, serum biochemical and cytomorphometric parameters of the sick Indian pangolin. As normal values for such parameters are largely unknown for wild population, the values from the present study represent a preliminary observation on haematological and biochemical analyses for Indian pangolins. The results of this study can be effectively utilize to evaluate red blood polymorphism and may be helpful in disease diagnosis in wild and captive Indian pangolins. The information of haematological parameters in pangolin still remains incomplete and inadequate. Future research works on long-term comprehensive studies to determine a baseline haematological and serum biochemical values will definitely throw more light for better understanding of these threatened animals.

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