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Research Article

EFFECT OF SEASON ON HAEMATOLOGICAL PARAMETERS OF CAPTIVE MUGGER CROCODILES (*CROCODYLUS PALUSTRIS*)

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ABSTRACT: The present study was undertaken to establish the effect of different seasons (winter, summer and rainy) on hematological parameters of captive mugger crocodiles (*Crocodylus palustris*) at Crocodile Conservation Park, Kotmi-Sonar, Chhattisgarh, India. The blood samples were collected by supra-occipital plexus and/or ventral tail vein puncture in 18 crocodiles each during winter, summer and rainy seasons. The findings of present study revealed a significant ($p<0.05$) increase in Total Erythrocyte Count (TEC) and Haemoglobin (Hb) values in summer season while the eosinophil and monocyte values were significantly ($p<0.05$) increased during winter season. Thrombocyte count was significantly ($p<0.05$) increased during rainy season as compared to winter and summer season. The present study has provided base line values for changes in haematological parameters in captive muggers.

Key words: Mugger crocodile, Captivity, Season, Haematology.

INTRODUCTION

Marsh crocodile or Mugger (*Crocodylus palustris*) is a widespread crocodilian species in Indian subcontinent. However, the population of this species is reportedly declining due to various causes like hunting for skin and indigenous medicine along with habitat destruction (Joshi *et al*. 2011). Mugger is considered to be a vulnerable species in the Red List of IUCN and is placed under schedule I in Wildlife Protection Act, 1972 (Choudhury and Chowdhury 1986). A Crocodile Conservation Park has been established in Munda pond at Kotmi Sonar of Janjgir-Champa District of Chhattisgarh, India to provide better protection to this species along with prevention of crocodile-human conflict. The Munda pond in Kotmi Sonar village is spread over an area of 85 acres and lies at 22.00'N Latitude and 82.02'E Longitude. The terrain of Kotmi Sonar is almost plain with gentle slope and is situated in 620 meters above mean sea level. In summer season, temperature of this area reaches up to about 50 ºC while it falls down to near about 8ºC in winter season. The area receives an average annual rainfall of 1200-1400 mm. Hence, the area experiences a tropical hot and humid climate (Bharos and Kanoje 2007).

Blood parameters play an important role in diagnosis as well as assessing progression and arriving at prognosis in clinical and research situations in reptiles. The potential application of reference values for some species of crocodiles serves as a basis for disease investigations (Milan *et al*. 2000, Lovely *et al*. 2007). Mature erythrocytes in reptiles appear as elongated oval with dense round nucleus while mature thrombocytes are elongated oval with dense oval nucleus. Lymphocytes are round to oval with dense round nuclei with a light blue cytoplasm and azurophilic granules may be present.

Monocytes are round with oval to irregularly indented nuclei and a finely granulated cytoplasm. Heterophils have an eccentric round to oval nuclei with distinct fusiform cytoplasmic granules. Eosinophils have a clear cytoplasm with round pink granules and a central or eccentric round, oval, elongated, or bilobed nuclei. Basophils contain metachromatic granules that frequently obscure the round nucleus. Degranulated basophils with distinct vacuoles

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are due to faulty in blood collection, delayed sample processing, or slide preparation (Stacy *et al.* 2011). The accuracy and reliability of the whole blood parameter analysis depends upon identification and control or elimination of variables that may affect these results. However, the usefulness of clinical pathology in Mugger crocodiles is restricted by the lack of reference ranges for haematological values (Stacey and Whitaker 2000). Blood parameters of reptiles are highly variable and are greatly influenced by seasonal variations. As information about seasonal variation on blood parameters is meagre for tropical muggers, this study was conducted to find out the possible effect of season on haematological parameters of captive mugger crocodiles at Crocodile Conservation Park, Kotmi-Sonar, Chhattisgarh, India.

MATERIALS AND METHODS

The crocodiles were captured by free catch method using nets and restrained manually by securing the jaws and limbs using ropes and tapping the eyes as per method suggested by Webb and Messel (1979). The animals were subjected to close inspection followed by physical examination to judge the body condition on the basis of thickness of the neck, muscles of the supra-temporal fossa and tail position (Huchzermeyer 2003).

Blood samples were collected from adult crocodiles (n=18) 9 male and 9 female each having good body condition during winter, rainy and summer season (August, 2016 to July, 2017) to discern seasonal alteration in haematological parameters. About 4 ml of blood sample was collected from ventral tail vein and/or supra-occipital plexus using a 20G sterile disposable syringe (Stein 1996). The blood samples were immediately transferred to properly labelled EDTA vials.

Blood smear was prepared by cover slip method from fresh whole blood, air dried and kept in sealed slide box and transported to laboratory for differential leukocyte count (DLC). The haematological parameters were recorded according to the technique described by Jain (1986). Haemoglobin (Hb) concentration was estimated by Sahli's Haemometer. Packed cell volume (PCV) was estimated by microhematocrit centrifuge method. Total erythrocyte count (TEC) and Total leucocyte count (TLC) was done with help of haemocytometer in Neubaur's counting chamber using both Natt and Herrick's solution and Shaw's avian solution as diluting fluid (Natt and Herick 1952, Otis 1974).

Mean corpuscular volume (MCV) was calculated by dividing the hematocrit (Hct) by the concentration of red blood cell count. MCV (fl) = {PCV(%)X10}/ TEC (millions/µl).

Table 1. Haematological values of *Crocodylus palustris* during different seasons (Mean ± SE).

Parameter	Season			
	Winter	Summer	Rainy	Normal Values (Stacey and Whitaker 2000)
Hb (g/dl)	7.85 ± 0.2^{b}	8.93 ± 0.24^{a}	8.37 ± 0.37^{ab}	8.3
PCV (%)	23.77 ± 0.78	25.52 ± 1.11	25.46 ± 1.16	24.9
TEC (millions/µl)	0.66 ± 0.02^{b}	0.78 ± 0.03^{a}	0.68 ± 0.01^{b}	0.69
MCV (fl)	357.96 ± 5.59^{ab}	328.65 ± 16.58^{b}	376.29 ± 10.47^{a}	326.43
MCH (pg)	118.33 ± 1.18	115.34 ± 5.63	123.69 ± 3.2	120.68
MCHC (g/dl)	33.08 ± 0.43	35.16 ± 0.83	32.89 ± 0.28	33.36
TLC (thousands/µl)	8.54 ± 0.45	9.1 ± 0.48	8.81 ± 0.51	8.71
Heterophils (%)	57.6 ± 0.35	58.1 ± 0.36	59.6 ± 0.39	5.6
Lymphocytes (%)	24 ± 0.05	25.3 ± 0.08	24.7± 0.07	2.48
Eosinophils (%)	6.2 ± 0.01^{a}	5 ± 0.01^{b}	5.1 ± 0.01^{b}	0.53
Monocytes (%)	1.1 ± 0.01^{a}	0.1 ± 0^{b}	0.1 ± 0^{b}	0.09
Basophils (%)	0.22 ± 0.14	0.28 ± 0.18	0.1 ± 0.01	0.01
Thrombocytes (thousands/µl)	19.90±0.20	20.17±0.26	20.82±0.18	20.9

*Values with different superscripts differ significantly ($p<0.05$) among the groups in different season.

Mean corpuscular hemoglobin (MCH) was calculated by dividing the hemoglobin by the red blood cell count. MCH (pg) = {Hb (g/dl) X10}/ TEC (millions/µl).

Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing the hemoglobin by the hematocrit. MCHC= {Hb(g/dl)X100}/ PCV (%). Blood smear was stained with Giemsa's stain followed by counting of cells for TLC under 100X objective of light microscope.

One Way Analysis of variance (ANOVA) was performed to assess whether haematological parameters varied significantly between and within the seasons with a Tukey's HSD means separation test to determine the differences among the means. Probability less than 0.05 ($p<0.05$) was considered as statistically significant. All statistical analyses were performed with SPSS 10.0 for Windows (SPSS Inc. Chicago, IL USA). All numerical data have been represented as the mean ± SE. (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Mean ± SE value of haematological parameters during winter, summer and rainy season in captive mugger crocodiles are presented in Table 1.

The values of haemoglobin (Hb) revealed significant ($p<0.05$) increase in summer season as compared to winter and rainy season. Higher Hb values during summer season has also been reported in reptiles by Duguy (1970), Sypik and Borysenko (1988) which might be due to higher environmental temperature leading to hemo-concentration and better nutritional status of the reptiles.

Packed cell volume (PCV) has been reported ranging from 20 to 40% in reptilian blood (Wallach and Boever 1983, Frye 1991). The findings of our study go in conformity with the findings of earlier workers indicating non-significant ($p>0.05$) alterations in PCV values in reptiles during winter, summer and rainy season.

A significant ($p<0.05$) increase in total erythrocyte count (TEC) values were recorded in summer as compared to winter and rainy season. However, the values were within normal range throughout the study. Sypik and Borysenko (1988) have reported TEC values ranging from 0.3 to 2.5 million/µl in reptiles. Highest TEC values have been reported before hibernation when food intake is more and the lowest immediately after hibernation due to utilisation of body fat reserve at the end of hibernation (Duguy 1970).

The values of mean corpuscular volume (MCV) increased significantly ($p>0.05$) in rainy season (macrocytosis) than winter and summer seasons. The values of mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) revealed non-significant ($p>0.05$) alterations (normochromic) during different seasons in our study and the values were within normal reference range as suggested by Milan *et al.* (2000) in salt water crocodiles (*Crocodylus porosus*).

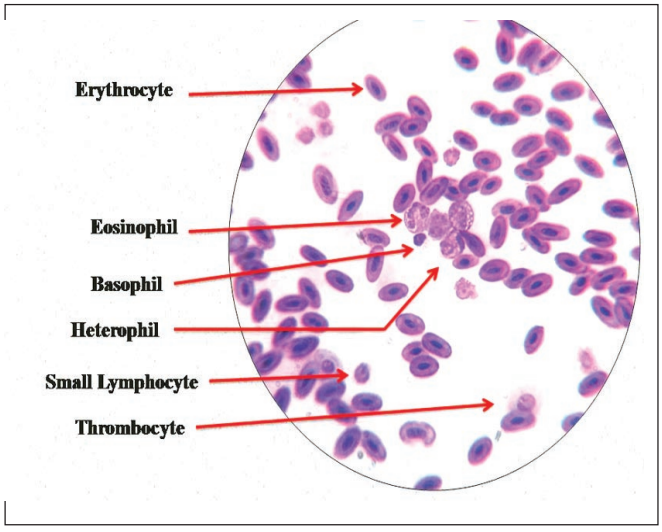

Fig. 1. Different blood cells in Mugger, Wright-Giemsa stain (40X).

The various types of blood cells observed during the study are represented in Fig. 1.

Total leucocyte count (TLC) values revealed non-significant ($p<0.05$) alterations throughout the study period. Mussart *et al.* (2006) also reported no significant variation in TLC values with respect to season and nutritional status in Caiman crocodile (*Caiman latirostris*). The findings of present study are also in agreement with findings of Lovely *et al.* (2007) reporting TLC values ranging from 3 to 26 X103/µl in farmed Nile crocodiles (*Crocodylus niloticus*).

A non-significant ($p>0.05$) alteration in heterophil values was recorded in different seasons during the study. However, the findings of our study are in concordance with Mussart *et al.* (2006) revealing non-significant alterations in heterophil values in Caimans during different seasons. In contrast to our findings, Duguy (1970) reported significant increase in heterophils in the peripheral blood during the summer and decrease during brumation in reptiles.

The values of lymphocyte count were non-significantly ($p>0.05$) higher in summer season as compared to winter and rainy season. A non-significantly higher lymphocyte count in summer season has been reported in Caiman crocodiles in summer and spring season as compared to

winter and autumn season (Mussart *et al*. 2006). However, decrease in circulating lymphocytes in captive tropical reptiles during the winter, despite lack of hibernation has been reported by Wright and Cooper (1981).

Eosinophil and monocyte values were increased significantly ($p<0.05$) during winter season as compared to summer and rainy season. Increased values of eosinophil and monocyte in winter season have also been reported by Mussart *et al*. (2006) in Caimans. Low numbers of eosinophils during the summer season and the highest values during brumation in reptiles has also been placed on record by Duguy (1970). In contrast to our findings, no seasonal alterations in monocyte count was recorded by Sypik and Borysenko (1988) and further explained that due to low number in blood film monocyte count is least affected by seasonal variation.

A non-significant ($p>0.05$) increase in basophil values was recorded in summer season as compared to winter and rainy season. The observations of Saint and Girons (1970) and Mussart *et al*. (2006) support the findings of our study that basophils are least affected by seasonal changes in crocodiles.

The values of thromobocytes were significantly ($p<0.05$) higher in summer and rainy season as compared to winter season. However, the values were within normal reference values ranging from 20 to 22 thousands/µl as reported earlier by Rajesh *et al*. (2013) in captive mugger crocodiles.

CONCLUSION

The findings of present study have provided a preliminary baseline data on haematological parameters in captive muggers which can prove to be useful in diagnostic investigations in mugger crocodiles. Future studies need to be conducted with some additional parameters and large sample sizes to arrive at species-specific range for mugger crocodiles and also to eliminate any other differences arising out of physiological or ecological conditions.

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