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### A study on haematological parameters of Indian spectacled cobra (*Naja naja*)

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### Abstract

A study was conducted on 12 captive Indian Spectacled Cobras (*Naja naja*) which underwent routine health screening procedures. The snakes were physically restrained and 2 ml of blood was collected aseptically from ventral coccygeal vein. Haematological examination was performed by manual counting method using Natt and Herrick's dilution fluid and using a blood gas analyser. The values of total erythrocyte count, volume of packed red cells, haemoglobin and differential leukocyte count values were similar to those obtained in other species of cobras. The data obtained may be useful as reference values for haematological parameters of Indian Spectacled Cobras.

Keywords: Haematology, Indian spectacled cobra, snake

### Introduction

Reptiles are one of the most diverse classes among all living creatures in the animal kingdom. They are poikilothermic animals with body temperature highly dependent on their surrounding environment. Though the interest in reptiles as a phylogenetically ancient group of vertebrates is an old one, there is noexhaustive information on their haematology. Reptiles, especially snakes, are rarely studied due to various reasons. Reptilian RBC's have nuclei unlike that of mammals. It has also been found that the blood composition in reptiles shows fluctuations depending on season and motor activity (Binyonand and Twigg, 1965; Lillywhite and Smits, 1984) <sup>[3, 8]</sup> phase of reproductivecycle (Acuna, 1974) <sup>[1]</sup> and the level of serum corticosterone (Saad, 1984) <sup>[12]</sup>. The haematological profile depends also on geophysical conditions of the habitat (Hutchison and Szarski, 1965) <sup>[6]</sup> and photoperiod (McLean, 1975; Ashby, 1985) <sup>[9, 2]</sup>. The number of cells in circulating blood is also determined by age, sex and health of the snake. The leukogram of reptiles continuously changes especially during stress and pathological states associated with parasitism (Saint Girons, 1960; Duguy, 1970) <sup>[13, 5]</sup>. The present study was conducted to assess the haematological parameters of Indian Spectacled Cobras which underwent routine health screening procedures.

### **Materials and Methods**

Twelve apparently healthy Indian Spectacled Cobras maintained at the Zoological Gardens, Thiruvananthapuram, which underwent routine health screening were included in the study. Individual snakes were segregated and housed in a separate enclosure for close observation of behaviour and also to assess health status. A thermo-hygrometer (HTC instuments<sup>TM</sup>, Maharashtra, India) was placed in the enclosure to monitor atmospheric temperature and humidity. All snakes were sexed using "cloacal probing" method.

### **Blood collection and analysis**

The snakes were physically restrained and 2 ml of blood was collected from the ventral coccygeal vein after aseptic preparation of the site using 2% chlorhexidine solution. A 24 G needle attached to a 2 ml syringe was inserted along the ventral midline at a 45° angle between two subcaudal scales approximately half the distance from the vent. The needle was inserted until it touched the vertebral column and moved slowly along it with simultaneous aspiration until blood was withdrawn into the syringe. Immediately after collection, a drop of blood was used for preparing blood smear by "push" smear technique for Differential Leukocyte Count (DLC). The rest of the blood sample was then transferred to K2 EDTA vials and was used for estimating total erythrocyte count and total leukocyte count by manual counting method using

Natt and Herrick's dilution fluid (Karthik, 2013) <sup>[7]</sup>. Volume of packed red cells and haemoglobin were estimated using a blood gas analyser (Epoc<sup>TM</sup> Blood Analysis System and Epoc BGEM Test Card, Epocal, INC. Ottawa, ON Canada). The blood collection procedure was carried out quickly to minimize stress associated with handling.

### Statistical analysis

The data obtained during the study was subjected to statistical analysis as described by Snedecor and Cochran (1994) <sup>[15]</sup> using the statistical software SPSS version 21.0. The value of P<0.05 was considered significant (Snedecor and Cochran, 1985). The results were expressed as Mean±Standard Error (Mean±SE).

### Results

Mean±SE value of temperature and relative humidity of the enclosure were  $89.83\pm0.56$  °F and  $84.07\pm0.50$  per cent, respectively. Out of the 12 snakes, eight were males and four were females. The mean±SE value of haemoglobin concentration was found to be  $7.60\pm0.29$  g/dL. The mean±SE value of TEC was  $5.88\pm0.27\times10^6$ . Total Leucocyte Count was found to be  $9.78\pm0.39\times10^3/\mu$ L. VPRC was  $19.85\pm1.07$  per cent. The mean±SE values of lymphocyte count was  $51.10\pm1.14$  per cent. The mean±SE value of heterophil count was  $45.30.\pm1.22$  per cent. The mean±SE value of monocyte count was  $1.90\pm0.076$  per cent. The mean±SE value of monocyte of basophil count was  $0.20\pm0.041$  per cent. All the

haematological parameters estimated are presented in the Table 1.

Table 1: Haematological values of Indian Spectacled Cobras

Parameter	Mean±SE
Haemoglobin Conc. (g/dL)	7.60±0.29
VPRC (%)	19.85±1.07
TEC (10 <sup>6</sup> /µL)	$0.588 \pm 0.27$
TLC (10 <sup>3</sup> / μL)	9.78±0.39
DLC (%)	
Lymphocytes	51.10±1.14
Heterophils	45.30.±1.22
Monocytes	1.90±0.076
Basophils	$1.50\pm0.084$
Eosonophils	$0.20\pm0.041$

### Discussion

The values obtained were compared with the previous studies (Table 2) and the results are discussed below. The value of haemoglobin concentration obtained in this study was in accordance with those obtained by Muliya and Bhat (2016) and Dissanayake *et al.* (2017)<sup>[4]</sup>, but slightly higher than the values obtained by Parida *et al.* (2014)<sup>[11]</sup>. The values were significantly higher than that of Monocellate Cobra, Siamese Spitting Cobra and Golden Spitting Cobra as noted by Salakij *et al.* (2012). The volume of packed red cells was similar to that reported by Salakij *et al.* (2012) but lower than that (2016)<sup>[10]</sup>.

Table 2: Comparative haematological values among cobras (Range)

Parameters	Monocellate Cobra, Siamese Spitting Cobra, Golden Spitting Cobra (Salakij <i>et al.</i> , 2012)	Indian Spectacled Cobra (Parida <i>et al.</i> , 2014) <sup>[11]</sup>	Wild Indian Spectacled Cobra (Muliya and Bhat, 2016) <sup>[10]</sup>	Wild population of Spectacled Cobra (Dissanayake <i>et al.</i> , 2017) <sup>[4]</sup>
Haemoglobin Conc. (g/dL)	4.8-6.5	6.5-6.9	7.9-8.5	6.6-7.6
VPRC (%)	18-21	25-29	32-33	20-23
TEC (10 <sup>6</sup> /µL)	0.57-0.65	0.34-0.39	1.1-1.3	0.49-0.58
TLC (10 <sup>3</sup> /µL)	9.8-14.3	11.7-12.1	9.8-10.8	11.9-12.4
DLC (%)				
Lymphocytes	66-71	47-49	48-56	64-66
Heterophils	27-31ª	23-25	41-49	36-41 <sup>a</sup>
Monocytes	0-1.2	2.5-4	0.4-1.0	4.6-5.6
Basophils	0-0.2	4.6-5.2	0.5-1.9	0-1.2
Eosonophils	0-1.4	2.6-3.6	0.1-0.5	2.1-2.9

a-Heterophils and Azurophils

Total erythrocyte count noted was in accordance with values obtained in Monocellate Cobra, Siamese Spitting Cobra and Golden Spitting Cobra (Salakij et al., 2012) and in wild population of Naja naja (Dissanayake et al., 2017)<sup>[4]</sup>. The value was more than that obtained by Parida et al. (2014)<sup>[11]</sup> and less than the count obtained by Muliya and Bhat (2016) <sup>[10]</sup>. Total leukocyte count was in accordance with study done by Salakij et al. (2012) and Muliya and Bhat (2016)<sup>[10]</sup>. The value was less when compared to the finding of Parida et al. (2014) <sup>[11]</sup> and Dissanayake et al. (2017) <sup>[4]</sup>. Lower lymphocyte count was seen in this study compared to Salakij et al. (2012) and Parida et al. (2014)<sup>[11]</sup>, but lymphocyte count was in agreement with the findings of Muliya and Bhat (2016)<sup>[10]</sup> and Dissanayake et al. (2017)<sup>[4]</sup>. Heterophil count in the present study was in accordance with the findings of Muliya and Bhat (2016)<sup>[10]</sup> and higher than the value obtained by Salakij et al. (2012), Parida et al. (2014) [11] and Dissanayake et al. (2017)<sup>[4]</sup>. Monocyte count obtained in this

study was higher than that observed by Salakij et al. (2012) and Muliya and Bhat (2016) <sup>[10]</sup> but lower than the value obtained by Parida et al. (2014)<sup>[11]</sup> and Dissanayake et al. (2017)<sup>[4]</sup>. Basophil count was similar to the count obtained by Muliya and Bhat (2016)<sup>[10]</sup> and less than the count obtained by Dissanayake et al. (2017)<sup>[4]</sup>. Eosinophils were similar to count obtained in study by Salakij et al. (2012) and Muliya and Bhat (2016)<sup>[10]</sup> and lower count was seen when compared to Parida et al. (2014)<sup>[11]</sup> and Dissanayake et al. (2017)<sup>[4]</sup>. The variation seen in the various haematological parameters might be due to motor activity (Binyon and Twigg, 1965; Lillywhite and Smits, 1984) <sup>[3, 8]</sup>, living conditions of the snake (Hutchison and Szarski, 1965)<sup>[6]</sup> or the level of serum corticosterone (Saad, 1984)<sup>[12]</sup>. The aforementioned values provide the information about haematological values of Indian Spectacled Cobra which aid in assessing the physiological health of the species discussed.

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