Indian Journal of Animal Sciences **92** (3): 302–305, March 2022/Article
https://doi.org/10.56093/ijans.v92i3.122258

Haematology and serum biochemistry parameters in chemically immobilized Manipur's Brow Antlered Deer or Sangai

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Received: 28 August 2021; Accepted: 7 December 2021

ABSTRACT

Haematology and serum biochemistry parameters were determined for apparently healthy Manipur's Brow Antlered Deer (4) or Sangai (*Rucervus eldii eldii*) maintained at the Conservation Breeding Centre, Langol, Manipur under semi-captive conditions. Venous blood samples were collected from the animals via the jugular vein puncture following immobilization using drug mixture of medetomidine and ketamine. Results were determined for haematological and serum biochemical parameters for the species and because of limited published information available results were compared with values obtained from captive reared Thamin (*Rucervus eldii thamin*) and captive/free ranging Red deer (*Cervus elaphus elaphus*). Higher total white blood cell, absolute lymphocyte and eosinophil, total bilirubin, lactate dehydrogenase were recorded in Manipur's Brow antler deer than Red deer, while serum biochemical parameters such as sodium, alanine aminotransferase, alkaline phosphatase and sodium were higher while creatinine, albumin, triglycerides and cholesterol were lower than Thamin deer. The values reported here can be used as a starting point for establishing blood parameters for clinically healthy adult Manipur's Brow Antlered Deer in India. To our knowledge, this is the first report of base-line blood values for the Manipur's Brow Antlered Deer.

Keywords: Endangered, Haematology, Health, Interpretation, Sangai, Serum biochemistry

Haemato-biochemical parameters are important for comparative physiology (Rosef *et al.* 2004) for assessing the health and nutritional status of the animal (Khan and Zafar 2005, Gupta *et al.* 2007, Etim *et al.* 2014). Though information on haematological and biochemical profile for variety of wild animals are available, limited information exists for Indian ungulates (Sahoo and Arora 2002, Sudhan *et al.* 2006, Gupta *et al.* 2007, Johns *et al.* 2018). This is attributed to permission restrictions for detailed studies on endangered species in the country; limited opportunities and small sample size (Arnemo *et al.* 1994) besides the fear of disruption of management and well-being of animal resulting from frequent and systematic sampling (Hawkey *et al.* 1984). Ideally, generating reference baseline values for a species for assessing health or disease requires sampling of large number of animals for meaningful interpretation of outcomes (Ryser-Degiorgis 2013). Manipur's brow-antlered deer (*Rucervus eldii eldii*) is an important deer species with a small free ranging population limited to Loktak Lake of Manipur, India (Hussain *et al.* 2006, Angom 2012). Though few ecological studies addressing conservation ecology, habitat ecology, distribution, population status, etc. have been carried out (Angom and Hussain 2013, Tuboi and Hussain 2018, Singh and Khare 2018), information on physiological data and health status is lacking (Singh *et al.* 2017).

Manipur's brow-antlered deer, locally known as 'Sangai is an endangered sub-species of Eld's deer endemic to the Keibul Lamjao National Park (KLNP) (latitude 24°26′N to 24°31′N and longitude 93°49′E to 93°52′E) (Fig. 1).

The park is the only remaining natural home of the last surviving population of Manipur's brow-antlered deer (Hussain *et al.* 2006). It receives protection under Schedule I of the Wildlife (Protection) Act, 1972; the IUCN Red list of threatened species has listed it as Endangered based on a species level assessment (Singh *et al.* 2017).

The study was undertaken to document baseline values for standard haematological and biochemical variables in Manipur's brow-antlered deer following immobilization with medetomidine-ketamine drug mixture in Manipur, India in the year 2020.

MATERIALS AND METHODS

Animal sampling: In 2020, four (two stags and two hinds) Manipur's brow-antlered deer in good body condition were selected for relocation from Conservation

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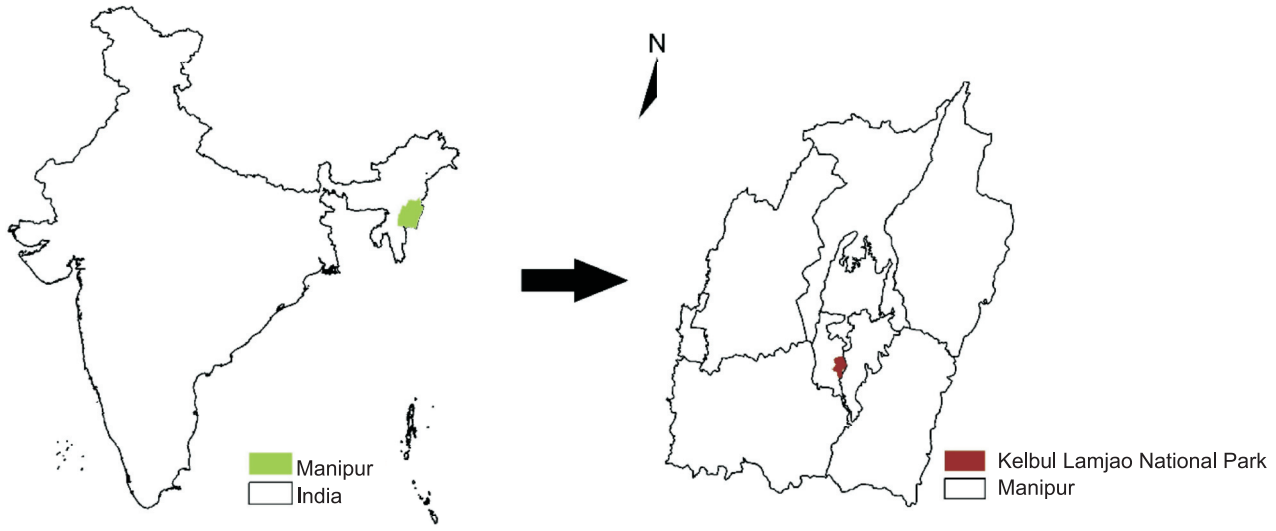

Fig. 1. Map showing Keibul Lamjao National Park (KLNP) in Manipur, India.

Breeding Centre, Langol, Manipur to Manipur Zoological garden, Iroisemba, Manipur for display purpose. The animals at the Conservation Breeding Centre were managed under semi-captive condition in a large enclosure with minimal human intervention. The animals were provided nutritional supplements occasionally including concentrate feed and mineral mixture and dewormed on biannual basis. The big enclosure was partitioned using temporary fence that supported confinement in smaller area and provided visual barrier. The animals were lured into smaller compartment using greens and concentrate as it would provide opportunity for darting from closer distances. Due consideration of the animal's health, condition, level of excitement, physiological status, sex, time of the day and ambient temperature were taken into account while calculating the drug dosages prior to animal darting. The identified individuals were remotely delivered with a drug mixture of medetomidine (70–90 µg/kg) and ketamine (1–2 mg/kg) using Dan Inject model-JM Syringe projector. The animals were left undisturbed following darting and approached after 10 to 18 min after assessing sedation safe for handling was achieved. Blood was collected within ten minutes of approach by jugular phlebotomy using a sterile syringe with an 18-gauge needle. Approximately 10–15 ml of blood was drawn aseptically. The animals were revived with atipamezole (0.4 mg/kg). The haematological and serum biochemical parameters were analysed within 6–8 h of blood collection based on standard methods (Jain 1986).

Necessary permissions for the field capture are accorded by the Government of India, MoEF&CC in accordance with the provisions under section 12 (bb)(i) and (c)(i) of the Wildlife (Protection) Act, 1972; Central Zoo Authority (CZA) and PCCF & CWLW, Government of Manipur and the Institute's Animal Ethics committee.

Haematological and serum biochemical parameters: Red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), platelets, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils, lymphocytes, eosinophils, monocytes and basophils were measured. The tests were performed using blood analyser (Erba Diagnostics, Inc. Miami, Fl, USA) that uses laser detection in the flow cytometer to measure size and internal complexity of cells based on light scatter at different angles and provides the cell count in each sample.

Serum biochemical parameters were analysed spectrophotometrically using commercial kits in automatic clinical chemistry analyzer (Eppend Transasia, Model XL 200). A total of 21 biochemical constituents were assessed to evaluate the lipid profile, kidney and liver function. These included measuring enzymatic activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine kinase (CK), values of total protein, albumin, globulin, urea, creatinine, total bilirubin, blood urea nitrogen, triglyceride, cholesterol and elements sodium, potassium, chloride, calcium, phosphorus and magnesium.

Statistical analysis: The parameters in blood from Manipur's brow-antlered deer ($n = 4$) were calculated using Microsoft Excel 2010 and SPSS software (IBM Corporation, USA) for mean and standard deviation.

RESULTS AND DISCUSSIONS

Manipur's brow-antlered deer (*Rucervus eldii eldii*) have been maintained in few captive facilities in India however the information on haematology and biochemical parameters is lacking. This is the first report documenting the hematology and serum biochemistry of Manipur's Brow Antlered Deer (*Rucervus eldii eldii*) form India. The only other report available is from adult (n=20) chemically immobilized captive Eld's Brow antlered deer or Thamin deer (*Rucervus eldii thamin*) (Nimitsuntiwong *et al.* 2000) subspecies.

Table 1. Haematological parameters of Manipur's Brow antlered deer (*Rucervus eldii eldii*)

Haematological parameters (unit)	Unit	Mean±SD
Red blood cell (RBC)	(× 10^6/µL)	7.56±0.54
White blood cell (WBC)	(× 10^3/µL)	8.78±2.12
Haemoglobin (Hb)	(g/dL)	10.77±0.92
Platelets	(×10^3/µL)	531.60±143.15
Haematocrit (HCT)	(%)	32.93±2.74
Mean corpuscular volume (MCV)	(fL)	44±1.58
Mean corpuscular haemoglobin (MCH)	(pg)	14.32±0.39
Mean corpuscular haemoglobin concentration (MCHC)	(g/dL)	32.63±0.50
Neutrophils	(10^3/µL)	3.58±1.81
Lymphocytes	(10^3/µL)	3.67±0.99
Monocytes	(10^3/µL)	0.31±0.14
Eosinophils	(10^3/µL)	1.24±0.44
Basophils	(10^3/µL)	0

Table 2. Biochemical parameters of Manipur's Brow antlered deer (*Rucervus eldii eldii*)

Bio-chemical parameters	Unit	Mean±SD
Aspartate aminotransferase (AST)	(U/L)	51±5.59
Alanine aminotransferase (ALT)	(U/L)	50.75±8.38
Alkaline phosphatase (AP)	(U/L)	391.50±224.479
Gamma-glutamyl transferase (GGT)	(U/L)	22.5±1.29
Lactate dehydrogenase (LDH)	(U/L)	947±145.74
Creatine kinase (CK)	(U/L)	317.45±113.41
Total protein (TP)	(g/dL)	5.8±0.67
Albumin	(g/dL)	2.22±0.12
Globulin	(g/dL)	3.57±0.65
Urea	(mg/dL)	44.97±5.20
Creatinine	(mg/dL)	1.73±0.29
Total bilirubin (TB)	(mg/dL)	0.18±0.03
Blood urea nitrogen (BUN)	(mg/dL)	20.75±2.21
Triglyceride	(mg/dL)	13.75±3.862
Cholesterol	(mg/dL)	62.25±15.33
Sodium (Na)	(mmol/L)	137±0.82
Potassium (K)	(mmol/L)	4.27±0.22
Chloride (Cl)	(mmol/L)	98.5±1.29
Calcium (Ca)	(mg/dL)	8.57±1.27
Phosphorus (P)	(mg/dL)	5.2±0.90
Magnesium(Mg)	(mg/dL)	1.6±0.43

The individual as well as median value of different haematological and bio-chemical values for Manipur's brow antlered deer is summarised in Tables 1 and 2.

The haematological values obtained in the study were compared with the values for Thamin (*Rucervus eldii thamin*) (Nimitsuntiwong *et al.* 2000) and Red deer (*Cervus elaphus elaphus*) (Peinado *et al.* 1999, Rosef *et al.* 2004, Topal *et al*. 2010, Miller 2015, Mariti *et al.* 2019).

The mean±SD values for RBC, Hb, Platelets, HCT, MCV, MCH, MCHC, neutrophils, monocytes and basophils obtained in the present study were consistent with the values reported for Red deer, while WBC, lymphocytes and eosinophil were higher (Peinado *et al.* 1999, Rosef *et al*. 2004, Topal *et al.* 2010, Miller 2015, Mariti *et al.* 2019). The mean values for Hb and PCV obtained in the current study were consistent with the only reported parameter values for Thamin deer (Nimitsuntiwong *et al.* 1999).

The mean±SD values for biochemical constituents in Manipur's brow-antlered deer, viz. AST, ALT, AP, GGT, CK, TP, Albumin, Globulin, urea, creatinine, BUN, triglycerides, cholesterol, Na, K, Cl, P, Mg, Ca were consistent with the findings in Red deer, while values of TB and LDH were higher (Rosef *et al*. 2004, Topal *et al.* 2010, Miller 2015). The values of AST, TP, Globulin, BUN, K, Cl, P, Ca, for Manipur's Brow antler deer were in tandem with the findings of Thamin deer however, ALT, AP and Na were higher while Creatinine, Albumin, Triglycerides and cholesterol were lower (Nimitsuntiwong *et al.* 1999).

The values of AP were much higher (721 IU/L) in one of the hinds as compared to others animals and were attributed to animal being gravid. Higher values of AP have also been reported in pregnant hinds (Soch *et al.* 2008). The said hind fawned after 19 days of immmobilization. The absolute Eosinophil count for all the four animals were higher, possibly due to parasitic infestation though the intrinsic factors as reported (Ovington and Behm 1997) cannot be ruled out. Lactate dehydrogenase (LDH) in general was higher in the present study as compared to other deer species however specific reasons could not be ascertained. Higher values of ALT, TP, globulin, BUN, Urea, K, Cl and Ca were recorded in one male in velvet and similar findings have been recorded by French *et al.* 1956.

The animals were maintained in semi captive condition with minimal human and veterinary interventions. This may lead to physiological, psychological and adaptive changes individually or in group as referred by Morgan and Tromborg (2007). The variation observed in haematological and biochemical parameters between Manipur's Brow antler deer, Thamin deer and Red deer may be attributed to variety of extrinsic (temperature, climatic conditions, living conditions, nutritional status, management and husbandry practices, prevalent parasitic load, stress of capture, etc.) and intrinsic factor (species specific) that are known to alter the values (Campbell 2012). Data from more number of animals are required for making meaningful interpretation.

Studies on health condition for Manipur's Brow antler deer are lacking. The present study was undertaken as part of the animal shifting programme and the blood samples collected during the field operations were used for establishing the base line values for select haematological and serum biochemical values. The values obtained under the study were unique and referenced for the first time for the subspecies. The hematology and serum biochemical values for Manipur's Brow antler deer were based on limited population and do not account for variations within sub-populations defined by age, sex, subspecies, variable management practices or extrinsic factors such as

instrumentation and blood collection techniques. Large sample size would be advantageous to make the results most reflective for the healthy population however the study provides base line values for the Manipur's Brow antler deer that can be used for interpreting health and disease.

ACKNOWLEDGEMENTS

The authors are grateful to Principal Chief Conservator of Forest and Chief Wildlife Warden, Government of Manipur; Director, Manipur Zoological Garden for according necessary permission to carry out the operation. The authors are also grateful to the Director and Dean of Wildlife Institute of India for support to carry out the study as a part of the Conservation Action Plan for Manipur's Brow Antlered Deer Project. Financial assistance from Compensatory Afforestation Fund Management and Planning Authority (CAMPA) for the study is thankfully acknowledged. We thank the project staff Endrea Moirangthem and Tennison Gurumayum and field assistants for their help in executing the programme. We are thankful to Dr. Bhubon Ningthoujam and Padma Diagnostic Laboratory, Imphal for helping us in laboratory analysis.

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