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No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,
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Ph: +91 9385339863 | www.threatenedtaxa.org

Email: sanjay@threatenedtaxa.org

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Cover: *Pipistrellus tenuis* recorded during the small mammalian fauna study, Manipur, India. © Uttam Saikia.



Hematological value of captive Asian Elephants *Elephas maximus* around Chitwan National Park, Sauraha, Nepal

Roshan Ghimire¹ , Sagar Regmi² , Rakshya Shrestha³ , Amir Sadaula⁴  & Janardan Dev Joshi⁵ 

¹⁻³ Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan 44200, Nepal.

⁴⁻⁵ National Trust for Nature Conservation, Sauraha, Chitwan 44200, Nepal.

¹ghimiroshon21@gmail.com (corresponding author), ²saregme@gmail.com, ³rakshya977@gmail.com, ⁴naturalamir@gmail.com, ⁵janardan1291@gmail.com

Abstract: Veterinary hematology serves as an important screening procedure to assess general health conditions, diagnosis, and treatment of disease. This study aims to interpret and establish a set of hematology reference ranges for Asian Elephants managed by private and government facilities in Nepal. Blood samples from 50 elephants around Chitwan National Park, Sauraha were collected and hematological parameters such as total erythrocyte count and total leukocyte count were determined. The results show that the majority of hematological values were in line with the values previously published by different authors. The mean erythrocyte and leukocyte counts were reported as $3.32 \pm 0.93 \times 10^6$ cell/ μ L and 10448 ± 335.49 cells/ μ L respectively. No sex-associated significant difference was observed in the case of total erythrocyte count, whereas total leukocyte counts varied significantly within sexes. Our findings revealed no significant difference in hematological parameters between government and privately owned elephants. The hematological values from our study can be used as reference values for assessing the health condition of elephants in Nepal. Further research work should be conducted to evaluate the factors affecting hematological parameters.

Keywords: Captive, erythrocyte count, free-ranging, hemocytometer, human-wildlife coexistence, Leukocyte count, mega-herbivore, Proboscidea, rouleaux.

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Ethical statement: All activities were performed under the ethical guidelines of NTNC, Sauraha, Nepal.

Author details: DR. ROSHAN GHIMIRE has completed his Bachelor's degree in Veterinary Science and Animal Husbandry from Agriculture and Forestry University (AFU), Nepal. He is currently pursuing his postgraduate studies at Oklahoma State University, USA. DR. SAGAR REGMI has also completed his Bachelor's degree majoring in Veterinary Science and Animal Husbandry from AFU, Nepal. He has participated in multiple research projects related to public health and veterinary science. DR. RAKSHYA SHRESTHA is also currently pursuing her postgraduate studies at Oklahoma State University, USA. DR. AMIR SADAULA and JANARDAN DEV JOSHI has been working with the National Trust for Nature Conservation, Sauraha with the goal to protect the wildlife and their habitat. He also conducts health camps at nearby areas performing nail trimming, vaccination and treatment of captive elephants.

Author contributions: Conceptualization: Roshan Ghimire, Sagar Regmi, Amir Sadaula. Methodology: Roshan Ghimire, Sagar Regmi, Rakshya Shrestha. Data analysis: Sagar Regmi, Roshan Ghimire. Writing-original draft: Sagar Regmi, Roshan Ghimire, Rakshya Shrestha. Reviewing and editing: Amir Sadaula, Sagar Regmi, Janardan Dev Joshi. All authors have agreed to the final version of the manuscript.

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INTRODUCTION

Asian Elephants *Elephas maximus* are the largest of all mammals in Nepal and are one of three species of elephants existing today under the order Proboscidea. Wild elephants in Nepal occur in four isolated populations — the eastern population in Koshi Tappu Wildlife Reserve and Jhapa district, the central population in Chitwan National Park and Parsa National Park, the western population in Bardia National Park and adjoining municipalities, and the far-western population in Suklaphanta National Park and adjoining municipalities (Pradhan et al 2008). Being a mega-herbivore and having long-range movements including dispersing behavior, there is frequent contact of wild elephants with human beings. So, there is a chance of human-wildlife interaction as wild elephants pose a problem to the local communities because of the destruction of private property, crop destruction, attack, and injury (Shrestha 2007). However, captive elephants in Nepal have restricted freedom and have no independent grazing time.

Captive (working) elephants are prone to various health problems including swelling of the eye by foreign body pricks, opacity of the cornea, lameness due to sole pricks, and contusion by hitting rocks and logs. Infection of the sole may occur due to injuries. Various equipment used while controlling the animals, and the pressure of Hauda, a seat to provide passengers a safe and comfortable ride on the back while carrying guests and other loads, and Gaddi, metallic objects with pointed ends to restrain elephants, can cause wounds. Ecto- and endo-parasitism are also a common problem seen in captive elephants. The major infectious diseases affecting the elephant are anthrax, hemorrhagic septicemia, foot and mouth disease, rabies, tuberculosis, tetanus, encephalo-myocarditis, pox, salmonellosis, and herpes virus infection. Other parasitic diseases affecting blood cells include babesiosis, anaplasmosis, trypanosomiasis, and ehrlichiosis (Miller et al. 2015). The majority of captive elephants in Nepal are found in and around Chitwan National Park and are used for patrolling and tourism purposes. As the majority of captive private elephants are used for tourism purposes, they are economically important, which increases the need for proper veterinary care to improve their health status. Hematology is defined as the study of components of blood (red blood cells (RBC), white blood cells (WBC), Platelets) for diagnosis and monitoring disease (Wolfrum 2010). There is a broad variation in how animals respond to captivity when managed under different conditions of

management practices. In general, captive elephants are raised under good management conditions like proper health care, good dietary plans, so they are often healthier than free-ranging wild elephants.

Knowing the normal hematological values plays a major role in the proper diagnosis, treatment, and interpretation of diseases. Precise hematological reference intervals and normal blood values are useful for evaluating the health status of animals, monitoring the course of the disease, proper diagnosis, and to know the treatment efficacy (Silva & Kuruwita 1993; Janyamethakul et al. 2017). Although normal hematological values exist for Asiatic Elephants (Nirmalan et al. 1967; Janyamethakul et al. 2017), they may not be relevant because these values are affected by different genetic and non-genetic factors. So, elephants under different geography or different conditions of feeding, and housing practices may differ in hematologic values. Stress due to daily duty and activity, clinical condition (diseased state), temperature, and sex can make significant differences in hematological values (Swenson 1984; Addass et al. 2012; Yaqub et al. 2013). As no major work has been done in Nepal till now to establish the hematological parameters for captive elephants, the study aimed to evaluate and devise a set of hematology reference ranges for Asian Elephants in Nepal used in the private sector as well as in the government sector.

MATERIALS AND METHODS

Study area

The study was conducted within Chitwan National Park (CNP) which was established in 1973 as the first national park in Nepal and listed as a World Heritage Site in 1984. It is situated in the sub-tropical lowlands of the Inner Terai at an elevation of about 150 m in south-central region of Nepal. Sal *Shorea robusta* trees cover about 70% of the national park, area and the buffer zone mostly consists of agricultural fields along with community forests.

Feeding, housing, and working routine

Captive elephants in Nepal have restricted freedom. Mahouts take the elephants to cut and collect grasses for fodder in the morning (0500–0700 h) and bring them back to the hattisar (place where elephants are kept). The elephants are then taken back to the jungle for grazing from 1000 h to 1600 h. Besides grazing and fodder, they are fed daily with 15 kg of unhusked rice, 1.5 kg molasses, 25 g of table salt, and 25 g of gram packed

in a bundle of succulent grass collectively called Kuchi. The elephants who have no specific allocated work are freed from chains to collect fodder in the morning and graze during the afternoon.

Blood sample collection

Elephants between 4 to 70 years of age were included in the study. The age of most elephants were known and the age of a few elephants was estimated by the mahouts. Blood samples from 50 elephants from around Chitwan National Park, Sauraha were collected from the auricular vein between 0700–0900 h. All elephants were kept under similar conditions (i.e., housing, feeding, exercise). None of the sampled elephants suffered from visible or known clinical health issues or had been diagnosed and treated for any health issues in the months prior to this study which would alter the blood parameters. Blood samples were divided into two separate tubes:

- 1) EDTA tube and
- 2) Serum tube in which the serum was separated by centrifugation at 1,500 rpm for 5 min.

The samples were submitted to NTNC-BCC molecular lab, Sauraha, Chitwan, and hematology was performed within two hours of blood collection. Total RBC count and WBC count were determined using hemocytometer (Neubaur Counting Chamber).

We performed RBC and WBC counts manually using a hemocytometer because blood cells in elephants are larger and rouleaux formation occurs in elephants' RBC which differ from human blood cells due to which an automated human hematology analyzer can lead to unreliable results (Dutton 2008).

Data analysis

Statistical analysis was done using SPSS Version 20. The reference interval with 95% confidence intervals for each parameter was calculated. P values from the student T-test were used to determine significant differences of blood parameters between males and females and comparison with feeding habit and exercise of animals. The level of statistical significance was set at $\alpha < 0.05$.

RESULTS AND DISCUSSIONS

From our study, the number of captive female elephants was found to be significantly greater ($n = 42$) than captive male elephants ($n = 8$) in Sauraha. The aggressive behavior of males makes them more difficult to control under captive conditions, and aggressiveness further increases during the musth period. In the private sector where elephants are primarily used for tourism purposes, only female elephants are kept because they are more docile. But in the government sector, a few male elephants are kept for patrolling purposes. Our study showed that reference hematological values fall within the range published by other authors for Asian Elephants (Janyamethakul et al. 2017).

From our study the average erythrocyte count in male elephant was found to be $(3.21 \pm 0.15) \times 10^6$ cells/ μL ranging from 2.40×10^6 cells/ μL – 3.16×10^6 cells/ μL . In the case of female elephants, the erythrocyte count ranges from 2.04×10^6 cells/ μL – 4.95×10^6 cells/ μL with an average of $(3.34 \pm 0.11) \times 10^6$ cells/ μL . No sex-associated significant difference was observed in elephants from our study. Our study also revealed that the privately owned elephants showed fairly low RBC close to, or at a level which can be judged to be slightly anemic, whilst none of the government owned elephants showed such low RBC levels. The range of the erythrocyte count in both male and female elephants during our study was in line with the values reported by Janyamethakul et al. (2017) and slightly lower than values reported by Debbie & Clausen (1975) in African Elephants. The mean value of erythrocyte was found in line with the values reported by Brown & White, (1980) but greater than the value reported by earlier researchers during their study (Lewis 1974; Woodford 1979; Gromadzka-Ostrowska 1988). Comparably the overall mean value of total erythrocyte count was found to be lower than the mean value reported by Young & Lombard (1967) in African Elephants. The red blood cells in African and Indian elephants are biconcave discs and are large, possibly larger than in any other mammal, and have a mean diameter (MD) slightly greater than 9 pm ($1\text{pm} = 1 \times 10^{-12}\text{m}$) (Brown & White 1980). The larger

Table 1. Reference range of hematology (total erythrocyte count and total leucocytes count values for sampled captive elephants in Sauraha for both sexes).

Parameters	Unit	Range (Male, n = 8)	Range (Female, n = 42)	All elephants (n = 50)
RBC count	$\times 10^6$ cells/ μL	2.40–3.16	2.04–4.95	2.04–4.95
WBC count	cells/ μL	8500–15500	7100–16750	7100–16750

Table 2. Total erythrocyte count and leukocyte count of all sampled captive elephants in Sauraha irrespective of sex (Mean±S.E.).

Parameters	Unit	All elephants (n = 50)
RBC count	$\times 10^6$ cells/ μ L	3.32±0.93
WBC count	cells/ μ L	10448±335.49
S.E.—Standard error		

Table 3. Effect of sex on hematology of sampled captive elephants in Sauraha (Mean±S.E.).

Parameters		Total RBC count ($\times 10^6$ cells/ μ L)	Total WBC count (cells/ μ L)
Sex	Male	3.21±0.15	12312.5±729.16
	Female	3.34±0.11	10092.86±351.60
P-value		0.607 ^{NS}	0.014*

*—showed significant difference of blood parameters between sexes (P <0.05) | NS—Not significant.

Table 4. Effect of age on hematology of sampled captive elephants in Sauraha (Mean±S.E.).

Parameters			Total RBC count ($\times 10^6$ cells/ μ L)	Total WBC count (cells/ μ L)
Age	Calf (0–4 years)	N = 2	3.20±0.03	10975.5±2475
	Juvenile (5–12 years)	N = 5	3.39±0.13	10790±712.99
	Sub-adult (13–20 years)	N = 12	3.72±0.19	9941.67±2398.75
	Adult (above 21 years)	N = 31	3.16±0.68	10554.84±248.44
P-value			0.013*	0.165 ^{NS}

*—showed significant difference of blood parameters between sexes (P <0.05) | NS—Not significant | N—No of elephants.

Table 5. Hematological parameters of sampled captive elephants in private and government facilities in Sauraha (Mean±S.E.).

Parameters			Total RBC count ($\times 10^6$ cells/ μ L)	Total WBC count (cells/ μ L)
Owner	Private	N = 27	3.14±0.12	9983.33±484.05
	Government	N = 23	3.54±0.14	10993.48±425.79
P-value			0.256 ^{NS}	0.023*

*—showed significant difference of blood parameters between sexes (P <0.05) | NS—Not significant | N—No. of elephants.

size of elephant red blood cells was further reported by Jarernsak Salakij et al. (2005) and Gromadzka-Ostrowska et al. (1988). Despite the large size, the total RBC count in elephants is lower than other mammals. Low erythrocyte count seen in elephants suggests that the erythrocytes are still in the primitive state compared with other mammals and have not attained the efficiency in the transportation of blood gases that results from a reduction in size to facilitate numerical increase (Nirmalan et al. 1967). The lower erythrocyte count in elephants than in other species was supported by values reported by Benjamin (1978) and Egbe-Nwiyi et al. (2000) in species like sheep, goats, cattle, dogs, cats as well as finding of Windberger (2003) in different mammalian species including horses and rabbits. Lewis (1974) also reported that the total erythrocyte value of elephants is lower than in humans. A significant effect of sex was observed in hematological values in numerous species (Etim et al. 2013). But our study showed no sex-associated significant difference in the erythrocyte

count. Janyamethakul et al. (2017) also found no sex-associated significant difference in total RBC count in Asian elephants. This finding was further supported by the findings of earlier researchers (Silva & Kuruwita 1993; Salakij et al. 2005).

Our study revealed the average leukocyte count in male elephants to be 12,312.5±729.16 cells/ μ L ranging from 8,500 cells/ μ L–15,500 cells/ μ L. In the case of female elephants, the total leukocyte count ranges from 7,100 cells/ μ L–16,750 cells/ μ L with an average of 10,092.86±351.60 cells/ μ L. Sex-associated significant difference was observed in elephants. The result of our study was in line with the findings of Janyamethakul et al. (2017) and Young & Lombard (1967). However, our mean value was lower than the value reported by Lewis (1974) and Brown & White (1980) in Indian elephants and by Debbie & Clausen (1975) in African elephants. Comparably, the mean value reported during our study was found to be greater than the value reported in African elephants (Woodford 1979). Our finding

Table 6. Age, sex, RBC count, and WBC count of sampled elephants during the study.

	Elephant's name	Owner	Sex	Age (in years)	RBC Count (× 10 ⁶ cell/ μ L)	WBC count (cells/ μ L)
1	Sudarkali	Private	F	55	2.64	8750
2	Champakali (Ramu)	Private	F	45	2.62	16750
3	Punamkali	Private	F	48	2.93	9200
4	Sherkali	Private	F	60	3.5	7350
5	Ekatakali	Private	F	35	3.1	9900
6	Sambrikali	Private	F	15	3.86	9000
7	Sonakali	Private	F	50	2.6	8400
8	Gulabkali	Private	F	20	3.67	9750
9	Selfiekali	Private	F	13	4.67	8900
10	Basantikali	Private	F	48	3.14	11050
11	Laxmikali	Private	F	20	3.51	7950
12	Champakali (Balram)	Private	F	52	3.7	11200
13	Marutikali	Private	F	50	2.6	13650
14	Champakali (Wildlife camp)	Private	F	52	2.6	12550
15	Bijulikali (Wildlife camp)	Private	F	18	3.17	11650
16	Bobkin (Rain forest)	Private	F	50	3.21	10900
17	Champakali (Jungle wildlife camp)	Private	F	45	2.04	9450
18	Ranikali (Forest Resort)	Private	F	45	2.72	8550
19	Rupakali	Private	F	50	2.74	7550
20	Shantikali	Private	F	65	2.77	7550
21	Suvakali	Private	F	15	4.52	7500
22	Champakali (Om Rijal)	Private	F	55	3.7	8100
23	Laxmikali (Bikash Mishra)	Private	F	50	3.05	7650
24	Tulsikali	Private	F	6	3.3	8400
25	Rajkali	Private	F	50	3.07	16050
26	Gulabkali (Bikash Mishra)	Private	F	20	2.82	8550
27	Dipendragaj	Private	M	52	2.4	13250
28	Sherprasad	CNP	M	15	3.14	15500
29	Sundarmala	CNP	F	70	3.16	13550
30	Sanochanchankali	CNP	F	59	2.96	10500
31	Sano Ramgaj	CNP	M	11	3.76	10950
32	Binayak Prasad	CNP	M	27	3.68	12650
33	Madigaj	CNP	M	8	3.2	11450
34	Prakritikali	CNP	F	15	3.82	9900
35	Maankali	NTNC	F	55	4.24	11000
36	Malekali	NTNC	F	70	4.5	11100
37	Rajagaj	NTNC	M	4	3.23	13450
38	Junkali	NTNC	F	50	3.01	11850
39	Luckygaj	NTNC	M	4	3.17	8500
40	Rampyari	CNP	F	60	2.95	9500
41	Ganeshkali	CNP	F	31	2.92	12100
42	Koshikali	CNP	F	31	2.83	11100
43	Krishnachandragaj	CNP	M	5	3.09	12750
44	Himanikali	CNP	F	18	3.5	8750
45	Simsimkali	CNP	F	5	3.6	10400
46	Devikali	CNP	F	50	4.7	7100
47	Karnalikali	CNP	F	21	3.06	10750
48	Loktantrakali	CNP	F	13	3.1	8450
49	Chintamankali	CNP	F	30	4.95	8150
50	Tamarkali	CNP	F	16	4.8	13400

CNP—Chitwan National Park (Government) | NTNC—National Trust For Nature Conservation (Government).

revealed a sex-associated significant difference ($p < 0.05$) in elephant WBC count which was in agreement with the reports given by Young & Lombard (1967) and Salakij et al. (2005).

A significant difference ($p < 0.05$) was reported in RBC counts among different age groups (i.e., calf, juvenile, sub-adult, and adult, respectively) of elephants during our study. The total leukocyte count of the elephants in the calf age group (age up to 5 years) was found greater than other age groups which is in agreement with findings reported by Nirmalan et al. (1967). However, total leukocyte count in other age groups was found to be similar. This finding was further supported by Niemuller et al. (1990) where he found that the total leukocyte count in Asian elephants was a constant overtime and was similar in the different age groups of elephants (Niemuller et al. 1990). The variation of parameters might be due to different lab errors like sample preparation and transportation, storage, and blood collection method. During our study, a non-significant increase in total leukocyte count was found in a pregnant elephant as opposed to a non-pregnant, non-lactating female elephant. However the high leukocyte count in the pregnant elephant was also reported by Ajitkumar et al. (2009).

The elephants sampled in our study kept under private facilities showed lower average and wider range on RBC and WBC counts compared to elephants within government facilities. The wider range determines higher variation on blood parameters among elephants managed under private facilities. Management practices like deworming, vaccination, and foot dipping are performed on regular intervals within government facilities under the supervision of licensed veterinarians. But the elephants under private facilities were treated and dewormed only at health camps organized by the government at irregular intervals. No specific study has been done to date comparing the blood parameters of elephants kept under private and government facilities in Nepal. Our study involved samples collected within the same season. So the effect of season on hematological value was not possible to determine. However Gromadzka-Ostrowska et al. (1988) reported a slight increase in white blood cell counts and lowered red blood cell counts during the winter season. The lower RBC counts in the winter season may be due to the non-availability of green fodder and a poor diet.

CONCLUSION AND RECOMMENDATIONS

No visible or known clinical health issues had been diagnosed in the sampled elephants; none of the study elephants had been treated for any health issues in the months before this study. Knowing normal hematological values is paramount for proper diagnosis of disease. Further standardization of these values is needed for an accurate diagnosis. Since elephant blood parameters are affected by different factors, further research should be conducted to evaluate the effects.

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