RH:Reference Intervals of Asian elephants in Myanmar

SEX DIFFERENCES IN THE REFERENCE INTERVALS OF HEALTH PARAMETERS IN SEMI-CAPTIVE ASIAN ELEPHANTS (ELEPHAS MAXIMUS) FROM MYANMAR.

Diogo J. Franco dos Santos, D.V.M., M.Sc., John Jackson, M.Phil., Htoo Htoo Aung, B.Vet.Med., U Kyaw Nyein, B.Vet.Med., Win Htut, B.Vet.Med. and Virpi Lummaa PhD

From the Department of Animal and Plant Sciences, Alfred Denny Building, University of Sheffield, Western Bank, Sheffield S10 2TN, UK (Franco dos Santos, Jackson); From the Myanma Timber Enterprise, Ministry of Natural Resources and Environmental Conservation , West Gyogone Forest Compound, Bayint Naung Road, Insein Township, Yangon, Myanmar; From the Department of Biology, University of Turku, 20014 Turku, Finland

Correspondence author: Diogo Santos

e-mail address: djfsantos1@sheffield.ac.uk / diogo.joao.santos@gmail.com

Abstract: The reference intervals of health parameters for wildlife populations are a valuable tool for veterinarians and conservationists to monitor the health status and viability of endangered species. Natural variation in the health of the long-lived Asian elephant (Elephas maximus) is poorly understood, particularly in relation to differences between males and females. Longitudinal health data was collected from clinical examination, hematology and serum chemistry over three years from 227 healthy individually-marked Asian elephants varying in age and sex. The study population was semi-captive and used in Myanmar's timber industry, but maintained natural feeding and breeding behaviour. Body condition score (BCS) and blood pressure were investigated in clinical examinations. Hematological parameters included hematocrit, hemoglobin, total white blood cell count (TWBC) and differential blood cell counts. Serum chemistry parameters included blood urea nitrogen (BUN), creatinine, total protein, albumin, globulins, aspartate aminotransferase (AST), alkaline phosphatase (ALKP), triglycerides, creatinine kinase (CK), glucose, calcium, potassium, sodium and chloride. To the knowledge of the authors, this is the first description of BCS in an elephant population outside of zoos, and of blood pressure in this species using a novel adaptation of the Wrap Cuff pressure monitor. Several differences between the sexes were observed, with females generally having higher BCS and triglycerides, and males displaying higher ALKP and glucose levels. This study provides important clinical tools that can be used to assess the health status and improve management in this endangered species.

Key Words: health parameter, conservation, physiology, population health, reference interval, Elephas maximus.

INTRODUCTION

In veterinary and human medicine, the reference intervals (RI) of hematological and serum health parameters are commonly used tools in disease diagnostics, and can determine sub-optimal health status and improve our understanding of the physiological changes in cases of disease.[11,21,48] Health surveys of animal populations conducted by veterinarians also aim to (1) establish baseline health values for a species or population, (2) monitor population health and its changes and (3) ensure population viability.[11,15,35] As such, they are a useful tool not only for veterinary medicine, but for in situ and ex situ conservation in endangered species.

Most health survey studies to-date have focused on humans and domestic species such as mice, rabbits and dogs.[22,23,28,56] In contrast, less data exists on natural populations, particularly in endangered species. This is problematic, because health survey values for domestic populations may not be representative of animals in the wild that are exposed to different pathogens, levels of exercise and nutrition.[35] Consequently, Deem at al.[15] proposed that reference values should also be determined for wild populations, focussing on hematology, serum chemistry, vitamin and mineral levels as well as surveys for infectious disease and chemical contaminants. However, the methodological challenges of conducting surveys in the wild has meant that currently only a few studies exist for animals living in their natural habitat.

Importantly, due to inherent physiological differences between the sexes, several studies have suggested that reference health values for males and females may also differ.[10,16,17,48] Health parameters can also change with age, and in particular differences have been found between the ontegenetic period, adulthood, and during senescence.[25,48,56,58] Thus, distinguishing animals based on their age and sex is crucial when establishing reference values for health parameters.

The aim of this study was to use a large population of semi-captive Asian elephants living in their natural habitat and maintaining their natural behaviour to (1) establish reference values for clinical examination, hematology and serum chemistry health parameters, and (2) establish if there are sex differences in the health parameters of this long-lived megaherbivore, for which males and females exhibit differences in life-history. The majority of previous studies on the health parameters of African elephants have focused on wild individuals, but in Asian elephants most studies have been conducted in captivity using only a small number of animals.[1,24,40,47,52,59] Myanmar is unique because it employs over 5000 Asian elephants in the timber industry; the largest captive population in the world of this endangered species. The animals work for a maximum of eight hours per day extracting logs but spend the rest of the time in comparable freedom, foraging in the forest and interacting unsupervised, and the population is considered to be semi-captive.[13] These timber elephants offer an opportunity to establish reference values for a large number of Asian elephants with known age, sex and history. Another key advantage is the possibility to collect repeated blood samples from each individual because they are tamed and trained. The current study therefore offers an interesting comparison to existing studies on domestic species and zoological collections, and provides a rare insight into what the reference values for clinical evaluation, hematology, and serum chemistry may be in wild Asian elephants.

Materials and Methods

Study population

The current study population is owned by the government-run Myanma Timber Enterprise, which has kept records of elephant births, deaths, wild-captures, and other events across the country for over 100 years.[27,50] These records enable access to animals of known and varying age, reproductive history, and with recent health diaries. Each animal has a unique ID number recorded in a logbook and permanently marked on its back. For captive-born individuals (82% in the present sample), exact ages are known from recorded birth dates, and for wild-caught animals age is estimated by experienced veterinarians using body size, temporal/buccal depressions, ear folds, pigmentation and tusk size.[3] Working elephants are organized into small units with 6 to 7 animals of different sexes. At the end of the working day, they are released into the forest where they forage and mate without human control.

Elephants were sampled in 2016, 2017, and 2018 from three logging agencies in the Sagaing Division, namely, Kawlin (23°46' N, 95°40' E) East Katha and West Katha (40°26' N, 79°58' W). Overall, this study includes 227 elephants, of which 85 were males and 142 were females. Their ages ranged from 4 to 72 years old; blood collection is impossible for calves younger than 5 years because they are not yet trained to be handled by humans. Repeated individual samples were collected three times a year, corresponding to three seasons in Myanmar, namely, the hot and dry season (January to May), monsoon season (June to September) and cold season (October to December). However, not all of the study elephants were available in every season; 58 elephants were only sampled in one of the fieldtrips, 50 in two, 25 in three, 17 in four, 19 in five, 22 in six, 17 in seven, 13 in eight and 5 in all nine fieldtrips over the three-year period.

Sample collection and analysis

To investigate hematological and serum chemistry levels, blood was collected from an ear vein using a Vacuette® (Greiner Bio-One, Kremsmünster, Austria, 4550) system and three different tubes, namely, EDTA, heparin and serum separator tubes. The blood tubes were refrigerated until analysis in the laboratory for a maximum of 24 hours. For serum chemistry, the samples were centrifuged, and sera was collected and frozen until analysis in a laboratory in Yangon using the IDEXX VetTest® (IDEXX, Westbrook, USA, 04092). The blood samples collected in EDTA were used to perform a manual count of leucocytes using Turk's solution. Differential leucocyte counts were performed manually using a blood smear stained with Romanowsky stain. Glucose levels were obtained using an ACCU-Chek® (Hoffmann–La Roche, Basel, Switzerland, 124) Aviva glucometer. It is noteworthy that glucose levels in blood samples are known to decline due to glycolysis during sample storage in other species[19], but in these analyses it was not possible to control for any differences between samples in the time from collection until analysis (maximum of 7/8 hours) and the raw glucose level values should therefore be interpreted with this in mind. Using samples stored in heparin, hematocrit, haemoglobin, sodium, potassium and chloride levels were obtained using a VetScan i-Stat® 1 (Abaxis, Union City, USA, 94587) with an E3+ cartridge. Blood pressure was collected using the Omron M6 Comfort IT (Omron, Kyoto, Japan, 617-0002) blood pressure monitor with an Intelli wrap cuff. The cuff was applied under the anal skin lap in an area where the diameter of the tail was more regular. The elephants were trained by their mahouts to accept tail handling and to keep the tail still.

To determine the body condition of each animal, the elephants were weighed to the nearest kg using Eziweigh 3000 scales.[13] When the scale was not available, body weight was estimated from the chest circumference and shoulder height using formulae for females and males from Chapman et al., 2016[12], which explain variation in the real body weight with an R^2 value of 0.87 for females and 0.94 for males in the current study population. In addition, the body condition score (BCS) was assessed for all animals using a table developed by Morfeld et al., 2016[39] that scores each elephant between 1 (very thin) and 5 (very fat), using the covering of fat on the ribs, pelvic bone and backbone as references.

Data analysis

The reference intervals (RIs) were established using the guidelines provided by the American Society for Veterinary Clinical Pathology (ASVCP).[21] All statistical analyses were carried out in the R statistical package.[43] The RIs were first calculated for the whole population, and subsequently by sex to test whether the reference intervals for each health parameter differed between males and females.

RIs and confidence intervals were calculated using the singleRefLimit function from the referenceIntervals package.[18] Only animals that appeared healthy during a clinical evaluation and with no record of recent illness were included in these calculations. The sample size used in each analysis varied because not all parameters were recorded in each season and year or for each individual, and because outliers were removed using Horn's method,[21] which determines outliers in a Box-Cox transformed dataset using Tukey interquartile fences. The normality of each parameter was assessed using Shapiro-Wilk tests. Reference intervals were then calculated following the recommendations from the ASVCP guidelines: for health parameters with over 120 observations, non-parametric methods were used, and for sample sizes less than 120 (i.e. when separating sexes), robust methods were used, namely, bootstrapping to estimate the location and spread of the data.[21] Reference intervals (RI) were calculated with 90% confidence intervals in all tests.

To test for differences between the sexes, both linear mixed-effects models (LMMs) and generalized linear mixed-effects models (GLMMs) were used depending on the health parameter distribution, implemented with the lmer and glmer functions from the lme4 package.[4] In each model, the health parameter was the response variable and the link function used depended on the parameter's distribution. The majority of the health parameters displayed a Gaussian distribution and were analysed with LMMs. However, eosinophils, ALKP, CK, AST and triglycerides were positively skewed, and were analysed using GLMMs, with a poisson family and a log link function (eosinophils, ALKP and CK), a negative binomial family for AST and a gamma family with a log link function for triglycerides. Sex was included as the main fixed factor of interest, and the age of each animal was controlled for by including age category (4 levels) as a fixed effect. The age categories were based on elephant life-history and working schedule and included: (1) young animals after taming and in the early stages of training (4 to 10 years old), which would still be dependent on their mothers and other herd members in the wild; (2) adolescent animals (10 to 20 years old) that are finishing training, beginning to work and reaching sexual maturity; (3) full working-age animals (20 to 50 years old) that are at peak reproductive ages; and (4) retired elephants (> 50 years old) with declining fertility and survival rates.[33] The models also accounted for variation due to collection location (three levels), season (three levels) and year (two levels) as fixed effect factors, and elephant ID number (to account for repeated samples from individuals across years and seasons) as an intercept-only random effect. Statistical significance was determined at the 95% confidence level.

RESULTS

Overall population

The results presented here establish reference health status values for Asian elephants managed in semi-captivity, in an effort to provide a valuable resource for veterinarians and conservationists. The frequency distributions of health parameters for the overall population are shown in Figure 1.

Regarding parameters obtained through clinical examination (Table 1), the BCS RI was 2-4 with a mean ± SD (standard deviation used for all parameters) of 3.2 ± 0.5; the first values obtained in a semi-captive elephant population. To the knowledge of the authors, this study is the first to describe blood pressure in Elephas maximus, with an RI for systolic pressure ranging between 99-166 mmHg with a mean of 134 ± 16 mmHg and the diastolic pressure with a RI of 67-127 mmHg and a mean of 96 ± 15 mmHg.

For hematological health parameters (Table 2), the most notable findings were for the monocytes (RI of 21-55% and mean of 38 ± 9%), which were the most abundant white blood cell, followed by the lymphocytes (RI of 16-46% with mean of 30 ± 8%). RIs were also obtained for hematocrit, hemoglobin, and total white blood cell count (Table 2).

Serum chemistry parameters are rarely measured in elephants, so the RIs provided in Table 3 provide a basis for the clinical evaluation of health in this species, particularly for triglycerides (RI of 0-58 mg/dL and mean of 15 ± 16 mg/dL) and CK (RI of 31-385 U/L and mean of 147±89 U/L). RIs were also obtained for blood urea nitrogen (BUN), creatinine, total protein, albumin, globulins, aspartate aminotransferase (AST), alkaline phosphatase (ALKP), glucose, calcium, potassium, sodium and chloride.

Sex differences in health parameters

Clinical examination

The frequency distributions of health parameters for males and females are presented in Figure 2. Asian elephant males are considerably larger than females, with the males in this study sample ranging in their body weight between 864 kg (4 years old) and 3601 kg (60 years old), compared to 894 kg - 3198 kg (5-39 years old) for females. The sexes also differed significantly regarding their Body Score Index (Table 1), with males displaying an overall BCS 6% lower than females (mean females = 3.4 ± 0.6, n = 467; mean males = 3.2 ± 0.5, n = 281; t = -2.640; P = 0.009). Systolic pressure was not significantly different between the sexes (mean = 134 ± 15, n = 137 and mean ± SD = 134 ± 17, n =105 for females and males, respectively; t = 0.322; P = 0.748). Similarly, no significant difference was observed in diastolic pressure (mean = 96 ± 13, n = 136, and mean = 97 ± 18, n =109 for females and males, respectively; t = 0.636; P = 0.527).

Hematology

Males and females did not differ significantly in their hematological health parameters (Table 2). The hematocrit levels (mean = 35 ± 3%, n = 487, and mean = 34 ± 3%, n = 278 for females and males, respectively; t = -1.281; P = 0.202), haemoglobin levels (mean = 11.8 ± 1.1 g/dl, n = 385, and mean = 11.5 ± 1.2 g/dl, n = 218 for females and males, respectively; t = -1.410; P = 0.160), TWBC (mean = 15.8 ± 3.9 $x10^9$/L, n = 467, and mean = 15.8 ± 3.7 x10^9/L, n = 282 for females and males, respectively; t =-1.033; P = 0.303), lymphocytes (mean = 30 ± 8%, n = 413, and mean = 30 ± 7%, n = 249 for females and males, respectively; t = -0.714; P = 0.476), monocytes (mean = 38 ± 9%, n = 418, and mean = 38 ± 8%, n = 250 for females and males, respectively; t = -0.304; P = 0.761), heterophil levels (mean = 27 ± 8%, n = 413, and mean = 28 ± 8%, n = 247 for females and males, respectively; t = 1.407; P = 0.161), and eosinophils (mean = 4 ± 3%, n = 416, and mean = 4 ± 3%, n = 250 for females and males, respectively; t = -0.901; P = 0.368) were not significantly different between males and females.

Serum chemistry

Significant sex differences were found for some of the serum chemistry health parameters (Table 3). Females had, on average, 5% lower glucose levels (mean = 4.0 ± 0.7 mmol/L, n = 491, and mean = 4.2 ± 0.8 mmol/L, n = 287 for females and males respectively; t = 3.111; P = 0.002), 19% lower ALKP levels (mean = 83 ± 37 U/L, n = 490, and mean = 102 ± 48 U/L, n = 289 for females and males, respectively; z = 3.956; P = <0.001), 30% higher triglyceride levels (mean = 17 ± 17 mg/dl, n = 498, and mean = 12 ± 13 mg/dl, n= 292 for females and males, respectively; t = -3.376; P <0.001) and 9% lower creatinine levels (mean = 1.0 ± 0.2 mg/dL, n = 477, and mean = 1.1 ± 0.2 mg/dL, n = 284 for females and males, respectively; t = 2.249; P = 0.026). In contrast, sex differences in BUN were only marginal (mean = 16 ± 5 mg/dL, n = 494, and mean = 16 ± 6 mg/dL, n = 290 for females and males, respectively; t = 1.949; P = 0.053), and total protein levels (mean = 7.8 ± 0.5 g/dl, n = 485, and mean = 7.6 ± 0.6 g/dl, n = 287 for females and males, respectively; t = -1.736; P = 0.084), albumin levels (mean = 3.1 ± 0.3 g/dl, n = 467, and mean = 3.0 ± 0.2 g/dl, n = 279 for females and males, respectively; t = -1.365; P = 0.174), globulins (mean = 4.8 ± 0.4 g/dL, n = 482, and mean = 4.6 ± 0.4 g/dL, n = 289 for females and males, respectively; t = -0.872; P = 0.384), calcium levels (mean = 9.95 ± 0.57 mg/dl, n = 484, and mean = 9.92 ± 0.54 mg/dl, n = 283 for females and males, respectively; t = -1.330; P = 0.186), potassium (mean = 4.7 ± 0.5 mEq/L, n = 383, and mean = 4.6 ± 0.4 mEq/L, n = 218 for females and males, respectively; t = -1.257; P = 0.209), sodium (mean = 129 ± 2.4 mEq/L, n = 376, and mean = 129 ± 2.3 mEq/L, n = 213 for females and males, respectively; t = -1.121; P = 0.264), chloride (mean = 91.1 ± 2.5 mEq/L, n = 376, and mean = 90.8 ± 2.3 mEq/L, n = 213; t = -1.066; P = 0.288), CK levels (mean = 147 ± 88 U/L, n = 479, and mean = 146 ± 90 U/L, n = 282 for females and males, respectively; z = 1.619; P = 0.105) and AST levels (mean = 19 ± 17 U/L, n = 493, and mean = 18 ± 18 U/L, n = 289 for females and males, respectively; z = -1.130; P = 0.258), were not significantly different for males and females.

DISCUSSION

The current study is the first to establish health parameter variation in a large population of Asian elephants managed in in their natural habitat. These results contribute to the small but much-needed group of studies focused on health variation in free-ranging systems. The health parameter variation observed in the current study is largely comparable with studies published on other species, and supports the need to differentiate animals according to their sex when considering typical health parameter values in a population.[14,24,38–41,45,47,52,54,55,57] Some differences e.g. in body score index (higher BCS in females) and serum chemistry parameters (lower creatinine, ALKP, glucose and higher triglycerides in females) were contrary to other Asian elephant populations managed in captivity, which highlights interesting potential differences arising as a result of management. For example, between-population differences may be explained by differences in exercise opportunities, disease exposure and foraging patterns between captive and wild/semi-captive animals. Importantly however, variation in health parameters may also be the result of differences in location, sample size, the collection and analysis methods used, the population's demography (sex and age), the duration of the study (including possible seasonal differences) and the life-history of the animal (working, pregnant, feeding babies, sick, etc).

Very few studies have successfully quantified the health variation of wild populations. To establish the reference values presented here, the recommendations of the American Society for Veterinary Clinical Pathology (ASVCP) were used.[21] Unfortunately, adhering to the recommended guidelines may often be challenged by the reality of field conditions, especially for endangered species in situ that often have small population sizes and occur in fragmented habitats. The current study on semi-captive animals provided a unique opportunity to obtain reference values that may be comparable to wild systems while adhering to ASVCP guidelines. First, the current population lives, feeds and mates in their natural environment with less human interference. Second, the use of timber elephants enabled the collection of repeated samples in more significant numbers, longitudinally, across several seasons, and without having to use anaesthesia (animals are trained). Third, the presence of accurate life-history information for each animal also allowed the study of health variation in relation to demographic variation. Finally, the detailed records for each individual enabled the selection of a healthy sample group for the analyses, avoiding animals that are a clinical concern to veterinarians overseeing the study elephants. Ultimately, establishing range values for health parameters in natural systems is crucial for veterinary and conservation management.

A key result from the analysis was the determination of range values for BCS, which informs us about the overall perceived nutritional condition of the population. This study is the first to the authors' knowledge to apply a BCS to elephants living and foraging independently in their natural environment without significant human provisioning. Consequently, the results presented differ from those obtained by the group that developed the BCS on zoo elephants in America, where most of the animals were very fat (4) or obese (5).[39] MTE elephants forage naturally in the forest with changes to food availability throughout the year, and their BCS was considerably lower than in American zoo elephants. Morfeld et al.[39] also presented results for the triglyceride level associated with each BCS score in American zoo elephants, comparing BCS to a major component of body fat content. Unfortunately, because they only present the average for each BCS score, it is not possible to compare their findings directly with this study.[39] Only one other study quantified triglycerides in Asian elephants, but this time using wild individuals from Sri Lanka. [51] Our results are comparable with the results obtained in the study on wild elephants, whereas the values obtained by Morfeld et al.[39] on zoo elephants are considerably higher. These results reinforce the utility of the timber elephant population as a model for wild elephants.

To the knowledge of the authors, systolic and diastolic blood pressure have not been described in elephants until now. The description of blood pressure in elephants was made possible using a novel adaptation of the Wrap Cuff pressure monitor, which was applied to the anal skin lap around the tail. High and low blood pressure is widely used as an indicator of health in the cardiovascular system, but also for the function of other organs such as the kidney, in both humans and non-human animals.[7,29] Although there are no other studies available to compare the range values for blood pressure in elephants with, these results are in accordance with range values described in humans, cats and dogs,[7,29,42] and provide a useful benchmark for veterinarians studying Asian elephants in the future.

The biggest differences to previous studies on Asian elephant health parameter values were in the differential white blood cell counts, especially between lymphocytes, monocytes and neutrophils. One reason for this may be confusion due to bilobed and sometimes trilobed cells that have been considered to be lymphocytes.[1,24,41,59] Silva & Kuruwita, 1993b[53] analysed these cells and saw that their granules were peroxidase positive and identical to the granulocyte (neutrophil and monocyte), but did not stain in Leishman-stain blood smears, like the non-bilobed monocyte. With the Leishman-stain the cytoplasmic granules of neutrophils are coloured, and so these cells could only be monocytes and not lymphocytes. Similar findings were described by Salakij et al., 2005,[47] where they used Sudan Black B, α-naphthyl acetate esterase and β-glucuronidase and realized that the bilobed and trilobed cells would stain very similarly to non-bilobed monocytes. This classification was adopted to distinguish between lymphocytes and monocytes. The results from this study are in accordance with the ones reported by Salakij et al.,[47] who reported more monocytes than lymphocytes in captive Asian elephants in Thailand. However, using the same distinction for lymphocytes and monocytes for Sri Lankan elephants, Silva & Kuruwita, 1993[52,53] consistently found more lymphocytes than monocytes, in opposition to the results in this study.

Reference intervals were established for a range of serum chemistry indices, which are of use for disease diagnostics, quantifying the general level of health, and understanding the physiology of this endangered species. Different range values were obtained here compared to other studies, stressing the importance of having different RIs for different populations. For example, BUN levels indicate urea nitrogen in the blood, a waste product of protein digestion in the liver, and higher BUN levels were found here than in all previous studies available in Asian elephants.[52,53,57] However, at present, it is assumed that this value represents the normal range for this population, despite the fact it that was not possible to do other kidney or liver pathology tests. In contrast, creatinine levels were lower here than in all other available studies, but as with BUN, since they are comparable or under the levels observed in other species such as the horse, they were considered normal.[2,36,52–54] Total protein levels in the blood are used to understand the composition of the structural and defensive proteins in the blood and were found to be in accordance with previous studies.[53,57] However, differences were found in the composition of these proteins.[40,52] A higher albumin:globulin ratio was observed than previously reported, and although the reasons for such differences are not clear, they could reflect between-population variation. CK was also investigated, which is indicative of muscle stress and damage. CK levels have been reported in one other study using a sample of captive elephants from the forest Department in India (including babies and not working adults) but were lower than in the current study.[55] The high level seen in the MTE population may be due to the fact that these individuals are working animals that experience a higher levels of both stress and muscular exertion.[20]

One interesting finding is the consistent difference between males and females in several health parameters. First, females displayed a higher (3.4) BCS than males (3.2), despite individuals of both sexes being considered healthy. The observation of higher BCSs in females was also made in the study on North American zoo elephants, where 27.1% females had a BCS of 4 (fat) and 48.2% of the females had a BCS of 5 (obese), compared to 47.8% of males with a BCS of 4 and 17.4% with a BCS of 5.[39] Males in our study also exhibited lower overall levels of triglycerides than females, which is also indicative of poorer body condition. This result is in line the results of Morfeld et al.[39] who found a good correlation between the body condition and serum triglycerides, except between BCS 2 and 3 due to small sample sizes. Furthermore, similar findings we made in a study conducted on wild and captive Asian elephants in Sri Lanka, where females had higher levels of triglycerides than males.[51] Some of the observed sex differences could be explained by the difference in life-history between males and females in the study population. In MTE elephants, females stop working when pregnancy is detected, and are given rest for 2 years after birth, while males only stop working when they are sick and need treatment. Adding to this, males are under the influence of testosterone that is responsible for an anabolic metabolism of fat, decreasing fat storage.[30,37] In older human females, the diminished influence of oestrogen due to menopause increases the risk of fat storage.[6] These findings are consistent with the results obtained in this study.

Regarding hematology, in other studies males have tended to have a higher red blood cell count, packed cell volume and haemoglobin level than females due to the positive effect of testosterone in erythropoiesis.[26,31,46] Surprisingly, no significant differences between males and females were found in haematocrit or haemoglobin level. Similarly, no significant sex differences in white blood cells were observed, contrary to some descriptions in other species.[8,9]

Concerning sex differences in serum chemistry levels, previous studies on other species (wild and lab populations) have reported mixed findings.[8,26,46,60] In the current study, no differences between the two sexes were observed in either the total protein, or any of its components. CK levels are predicted to be higher in males than in females due to an increase in muscular activity and muscle mass in male elephants, but in our study, no differences were found.[55] In contrast, females displayed lower levels of ALKP and glucose, which is in line with other studies, mainly in humans, but the physiological mechanisms and the reasons for this difference remain unknown.[44,49] In the case of creatinine, females have lower values than males. Because creatinine is a metabolite from processes in the muscles, one may expect that males have higher levels of creatine due to increased muscle mass. Adding to this, given that the excretion rate of creatine is constant, it is expected that males with a higher body mass have higher creatinine levels.[5]

Overall, the sex differences in health parameters observed mirror the differences in body size, behaviour and life-history between male and female Asian elephants. Male elephants do not reach peak reproduction until their 30s or 40s [34] and experience a significantly higher mortality risk across all ages.[32] These sex differences may also have important implications for the clinical examination of this species. Specifically, normal health measure values in females may actually represent a clinical signal in males, and vice versa. Thus, it is crucial to include sex differences when considering physiological status in conservation and population management.

In conclusion, the current study establishes baseline reference intervals for physical examination, serum chemistry and hematology in a large semi-captive population of Asian elephants managed in their natural habitat. The reference values obtained in this study provide a useful tool not only for MTE veterinarians, but for use the in situ and ex situ management of this endangered species. The findings in this study also highlight the need for caution when comparing results between species, but even between populations of the same species and between the sexes. Understanding health parameter variation in free ranging populations can be used as a clinical tool to assess the health status of animals, working towards providing better management of species in the wild and in captivity.

Acknowledgments: The authors would like to thank the ministry of Natural Resources and Environmental Conservation and the Myanma Timber Enterprise for the permission and help in developing the project. The authors would like to thank Crown Veterinary Resources staff and director Dr. Tint Naing for all the help provided in analysing the serum samples. The authors would like to thank Khin Than Win, Thu Zar Win, Mu Mu Thein and Ohn Mar Win for all the help in field logistics. The authors would also like to thank San Win, Thein Zaw Win, Sophie Reichert, Vérane Berger, Carly Lynsdale and Robin Cristofari for all the help in collecting and analysing samples in the field.

REFERENCES

1. Allen JL, Jacobson ER, Harvey JW, Boyce W. Hematologic and serum chemical values for young African elephants (Loxodonta african) with variations for sex and age. J Zoo Anim Med. 1985;16(3):98–101.

2. Aoki T, Ishii M. Hematological and Biochemical Profiles in Peripartum Mares and Neonatal Foals (Heavy Draft Horse). J Equine Vet Sci [Internet]. W.B. Saunders; 2012 [cited 2018 Jun 4];32(3):170–176. Available from: https://www.sciencedirect.com/science/article/pii/S0737080611005041

3. Arivazhagan C, Sukumar R. Constructing Age Structures of Asian Elephant Populations: A Comparison of Two Field Methods of Age Estimation. GAJAH J Asian Elephant Spec Gr Number [Internet]. 2008 [cited 2018 Jun 19];29:11–16. Available from: http://www.asesg.org/PDFfiles/2009/33121-Gajah-29.indd.pdf#page=15

4. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw [Internet]. 2015 [cited 2018 Jun 20];67(1):1–48. Available from: http://www.jstatsoft.org/v67/i01/

5. Braun JP, Lefebvre HP, Watson ADJ. Creatinine in the Dog: A Review. Vet Clin Pathol [Internet]. John Wiley & Sons, Ltd (10.1111); 2003 [cited 2019 Mar 13];32(4):162–179. Available from: http://doi.wiley.com/10.1111/j.1939-165X.2003.tb00332.x

6. Brown LM, Clegg DJ. Central effects of estradiol in the regulation of food intake, body weight, and adiposity. J Steroid Biochem Mol Biol [Internet]. Pergamon; 2010 [cited 2018 Jun 5];122(1–3):65–73. Available from: https://www.sciencedirect.com/science/article/pii/S0960076009002982

7. Brown S, Atkins C, Bagley R, Carr A, Cowgill L, Davidson M, Egner B,

Elliott J, Henik R, Labato M, Littman M, Polzin D, Ross L, Snyder P, Stepien R. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med [Internet]. Wiley/Blackwell (10.1111); 2007 [cited 2018 May 31];21(3):542–558. Available from: http://doi.wiley.com/10.1111/j.1939-1676.2007.tb03005.x

8. Bush M, Custer RS, Whitla JC. Hematology and Serum Chemistry Profiles for Giraffes (Giraffa camelopardalis): Variations with Sex, Age, and Restraint. Source J Zoo Anim Med [Internet]. 1980 [cited 2018 Jun 6];11(4):122–129. Available from: http://www.jstor.org/stable/20094493

9. Bush M, Custer RS, Whitla JC, Montali RJ. Hematologic and Serum Chemistry Values of Captive Scimitar-Horned Oryx (Oryx tao): Variations with Age and Sex. Source J Zoo Anim Med [Internet]. 1983 [cited 2018 Jun 6];14(2):51–55. Available from: http://www.jstor.org/stable/20094637

10. Bush M, Smith E, Custer R. Hematology and serum chemistry values for captive Dorcas gazelles: variations with sex, age and health status. J Wildl Dis [Internet]. 1981;17(1):135–143. Available from: http://www.jwildlifedis.org/content/17/1/135.short

11. Catenacci LS, Nascimento A, Muniz-Neta ES, Cassano CR, Deem SL, da Rosa EST, Parker P, Munhoz AD. First record of hematologic values in free-living and captive maned sloths (Bradypus torquatus ; xenartha, bradypodidae). J Zoo Wildl Med [Internet]. 2017 [cited 2018 Jan 26];48(2):312–318. Available from: http://zoowildlifejournal.com/doi/pdf/10.1638/2016-0025R1.1

12. Chapman SN, Mumby HS, Crawley JAH, Mar KU, Htut W, Soe AT, Aung HH, Lummaa V. How big is it really? Aeing the efficacy of indirect estimates of body size in Asian elephants. PLoS One [Internet]. 2016;11(3). Available from:

http://dx.doi.org/10.1371/journal.pone.0150533

13. Crawley JAH, Mumby HS, Chapman SN, Lahdenperä M, Mar KU, Htut W, Thura Soe A, Aung HH, Lummaa V. Is bigger better? The relationship between size and reproduction in female Asian elephants. J Evol Biol [Internet]. Wiley/Blackwell (10.1111); 2017 [cited 2018 Jun 20];30(10):1836–1845. Available from: http://doi.wiley.com/10.1111/jeb.13143

14. Dangolla A, Malitha AG, Silva I. Mineral Status in Blood Serum of Domesticated Elephants (Elephas Maximus) and Certain Plants of Sri Lanka. Vet Br Zoos' Print J. 2004;19(7):1549–1550.

15. Deem SL, Karesh WB, Weisman W, Deem SL, Karesh WB, Weisman W. Society for Conservation Biology Putting Theory into Practice : Wildlife Health in Conservation Linked references are available on JSTOR for this article : Putting Theory into Practice : Wildlife Health in Conservation. 2001;15(5):1224–1233.

16. Etim NN, Williams ME, Akpabio U, Offiong EEA. Haematological Parameters and Factors Affecting Their Values. Agric Sci [Internet]. 2014;2(1):37–47. Available from: http://www.todayscience.org/asarticle.php?paper_id=447100022

17. Fairbrother A, Craig MA, Alker K, Loughlin O. Changes in Mallard (Anas Platyrhynchos), Serum Chemistry due to age, sex, and reproductive condition. J Wildl Dis. 1990;26(1):67–77.

18. Finnegan D. referenceIntervals. 2014; Available from: https://cran.r-project.org/package=referenceIntervals

19. Fobker M. Stability of glucose in plasma with different anticoagulants. Clin Chem Lab Med [Internet]. De Gruyter; 2014 [cited 2019 Feb 28];52(7):1057–1060. Available from: https://www.degruyter.com/view/j/cclm.2014.52.issue-7/cclm-2013-1049/cclm-2013-1049.xml

20. Fowler ME, Mikota SK. Biology, Medicine and Surgery of Elephants. first edit. Fowler ME, Mikota SK (eds.). Ames, Iowa, USA: Blackwell Publishing; 2006. 1-596 p.

21. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol. 2012;41(4):441–453.

22. Fukuda S, Kawashima N, Iida H, Aoki J, Tokita K. Age Dependency of Haematological Values and Concentrations of Serum Biochemical Constituents in Normal Beagles from 1 to 14 Years of Age. Japanese J Vet Sci [Internet]. 1989 [cited 2018 Feb 7];51(3):636–641. Available from: https://www.jstage.jst.go.jp/article/jvms1939/51/3/51_3_636/_pdf/-char/en

23. Fukuda S, Tsuchikura S, Iida H. Age-Related Changes in Blood Pressure, Hematological Values, Concentrations of Serum Biochemical Constituents and Weights of Organs in the SHR/Izm, SHRSP/Izm and WKY/Izm. Exp Anim [Internet]. 2004 [cited 2018 Jan 26];53(2):67–72. Available from: https://www.jstage.jst.go.jp/article/expanim/53/1/53_1_67/_pdf

24. Gromadzka-Ostrowska J, Jakubow K, Zalewska B, Krzywicki Z. Haematological and Biochemical Studies in Female Domesticated Indian Elephants (Elephas maximus L). Comp Biochem Physiol. 1988;89A(3):313–315.

25. Horning M, Trillmich F. Development of Hemoglobin, Hematocrit, and Erythrocyte Values in Galapagos Fur Seals. Mar Mammal Sci [Internet]. 1997;13(1):100–113. Available from: http://doi.wiley.com/10.1111/j.1748-7692.1997.tb00614.x

26. Howell S, Hoffman K, Bartel L, Schwandt M, Morris J, Fritz J. Normal

hematologic and serum clinical chemistry values for captive chimpanzees (Pan troglodytes). Comp Med. 2003;53(4):413–423.

27. Jackson J, Childs DZ, Mar KU, Htut W, Lummaa V. Long-term trends in wild-capture and population dynamics point to an uncertain future for captive elephants. Proc R Soc B Biol Sci. 2019;

28. Jeklova E, Leva L, Knotigova P, Faldyna M. Age-related changes in selected haematology parameters in rabbits. Res Vet Sci [Internet]. 2009 [cited 2018 Jan 26];86:525–528. Available from: https://ac.els-cdn.com/S0034528808002245/1-s2.0-S0034528808002245-main.pdf?_tid=5c6cdac0-02a0-11e8-bf11-00000aab0f27&acdnat=1516975020_693ee9291d7f5226f14c108b0a20b0b2

29. Kannel WB. Blood Pressure as a Cardiovascular Risk Factor. JAMA [Internet]. American Medical Association; 1996 [cited 2018 May 31];275(20):1571. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.1996.03530440051036

30. Kaufman JM, Vermeulen A. The Decline of Androgen Levels in Elderly Men and Its Clinical and Therapeutic Implications. Endocr Rev [Internet]. 2005 [cited 2018 Jun 5];26(6):833–876. Available from: https://watermark.silverchair.com/edrv0833.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAbowggG2BgkqhkiG9w0BBwagggGnMIIBowIBADCCAZwGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQM-VomkeFEcxCC3Rl5AgEQgIIBbf0SoTzyrLIrEy0sZIryvLssMJ8iJAZmuzB3YGTMtzNKXf

31. Kelani OL, Durotoye LA. Haematological responses of the African giant rat (Cricetomis gambianus) to castration and androgen replacement. Vet Arh [Internet]. 2002 [cited 2018 Jun 5];72(1):39–49. Available from:

http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.446.4278&rep=rep1&type=pdf

32. Lahdenperä M, Mar KU, Courtiol A, Lummaa V. Differences in age-specific mortality between wild-caught and captive-born Asian elephants. Nat Commun [Internet]. Nature Publishing Group; 2018 [cited 2018 Aug 21];9(1):3023. Available from: http://www.nature.com/articles/s41467-018-05515-8

33. Lahdenperä M, Mar KU, Lummaa V. Reproductive cessation and post-reproductive lifespan in Asian elephants and pre-industrial humans. Front Zool [Internet]. 2014 [cited 2018 Jun 20];11(54):1–14. Available from: http://www.frontiersinzoology.com/content/11/1/54

34. Lincoln GA, Ratnasooriya WD. Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator. J Reprod Fertil [Internet]. 1996 [cited 2018 Aug 9];108:107–113. Available from: http://www.reproduction-online.org/content/108/1/107.full.pdf

35. López J, Waters M, Routh A, Rakotonanahary TF, Woolaver L, Thomasson A, Holmes E, Steinmetz HW. Hematology and Plasma Chemistry of the Ploughshare Tortoise (Astrochelys Yniphora) in a Captive Breeding Program. J Zoo Wildl Med [Internet]. 2017 [cited 2018 Jan 26];48(1):102–115. Available from: http://zoowildlifejournal.com/doi/pdf/10.1638/2016-0201.1

36. Lumsden JH, Rowe R, Mullen K. Hematology and Biochemistry Reference Values for the Light Horse. Can J Comp Med Rev Can Med Comp [Internet]. 1980 [cited 2018 Jun 4];44(1):32–42. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1320032/pdf/compmed00021-0036.pdf

37. De Maddalena C, Vodo S, Petroni A, Aloisi AM. Impact of testosterone on body fat composition. J Cell Physiol [Internet]. Wiley-Blackwell; 2012 [cited 2018 Jun

5];227(12):3744–3748. Available from: http://doi.wiley.com/10.1002/jcp.24096

38. Mel RK De, Weerakoon DK. comparative haematological analysis of Asian Elephants Elephas maximus Linnaeus, 1758 (Mammalia: Proboscidea: Elephantidae) managed under different captive. J Threat Taxa [Internet]. 2014;6(July):6148–6150. Available from: http://www.threatenedtaxa.in/index.php/JoTT/article/view/1599

39. Morfeld KA, Meehan CL, Hogan JN, Brown JL. Assessment of body condition in African (Loxodonta africana) and Asian (Elephas maximus) elephants in North American zoos and management practices associated with high body condition scores. PLoS One. 2016;11(7):1–20.

40. Niemuller C, Gentry P., Liptrap R. Longitudinal study of haematological and biochemical constituents in blood of the Asian elephant (Elephas maximus). Comp Biochem Physiol Part A Physiol [Internet]. 1990;96(1):131–134. Available from: http://linkinghub.elsevier.com/retrieve/pii/030096299090053U

41. Nirmalan G, Nair SG, Simon KJ. Hematology of the Indian Elephant. Can J Physiol Pharmacol. 1967;45(1):985–991.

42. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill, M.N., Jones DW, Kurtz T, Sheps SG, Roccella EJ. Cardiovascular outcome in white-coat versus sustained mild hypertension: a 10-year follow-up study. Circulation [Internet]. American Heart Association, Inc.; 2005 [cited 2018 May 31];111:697–716. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9799210

43. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.

44. Rahmioglu N, Andrew T, Cherkas L, Surdulescu G, Swaminathan R, Spector T, Ahmadi KR. Epidemiology and Genetic Epidemiology of the Liver Function Test Proteins. Sorensen TIA (ed.). PLoS One [Internet]. Public Library of Science; 2009

[cited 2019 Mar 13];4(2):e4435. Available from: https://dx.plos.org/10.1371/journal.pone.0004435

45. Ratnasooriya WD, Gunasekera MB, Goonesekere NCW. Serum glucose levels in captive Sri Lankan elephants. 1999;127:127–134.

46. Riviello MC, Wirz a. Haematology and blood chemistry of Cebus apella in relation to sex and age. J Med Primatol. 2001;30(6):308–312.

47. Salakij J, Salakij C, Narkkong N-A, Apibal S, Suthunmapinuntra P, Rattanakukuprakarn J, Nunklang G, Yindee M. Hematology, cytochemistry and ultrastructure of blood cells from Asian elephant (Elephas maximus). Kasetsart J (Natural Sci. 2005;39:482–493.

48. dos Santos Schmidt EM, Paulillo AC, Santin E, Dittrich RL, Gonçalves De Oliveira E. Hematological and Serum Chemistry Values for the Ring-necked Pheasant (Phasianus colchicus): Variation with Sex and Age. Int J Poult Sci [Internet]. 2007 [cited 2018 Jan 26];6(2):137–139. Available from: http://free-journal.umm.ac.id/files/file/Hematological and Serum Chemistry Values for the Ring-necked.pdf

49. Schwartz JB. The Current State of Knowledge on Age, Sex, and Their Interactions on Clinical Pharmacology. Clin Pharmacol Ther [Internet]. John Wiley & Sons, Ltd; 2007 [cited 2019 Mar 13];82(1):87–96. Available from: http://doi.wiley.com/10.1038/sj.clpt.6100226

50. Seltmann MW, Helle S, Adams MJ, Mar KU, Lahdenperä M. Evaluating the personality structure of semi-captive Asian elephants living in their natural habitat. R Soc Open Sci [Internet]. 2018 [cited 2018 Jun 22];5(172026). Available from: http://dx.doi.org/10.1098/rsos.172026

51. Silva I, Dangolla A. Blood levels of cholesterol and triglycerides in wild and

domesticated Asian elephants (Elephas. maximus) in Sri Lanka. Gajah. 2002;21:53–55.

52. Silva ID, Kuruwita VY. Hematology , Plasma , and Serum Biochemistry Values in Domesticated Elephants (Elephas maximus ceylonicus) in Sri Lanka. J Zoo Wildl Med. 1993;24(4):440–444.

53. Silva ID, Kuruwita VY. Hematology , Plasma , and Serum Biochemistry Values in Free-Ranging Elephants (Elephas maximus ceylonicus) in Sri Lanka. J Zoo Wildl Med. 1993;24(4):434–439.

54. van Sonsbeek GR, van der Kolk JH, van Leeuwen JPTM, Schaftenaar W. Preliminary validation of assays to measure parameters of calcium metabolism in captive asian and african elephants in Western Europe. J Vet Diagnostic Investig. 2011;23(3):504–511.

55. Sreekumar KP, Nirmalan G. Normal values for certain serum enzymes of clinical value in indian elephants. Vet Res Commun. 1992;16(6):411–414.

56. Touitou Y, Touitou C, Bogdan A. Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit, and erythrocyte counts. Clin Chem. 1986;32(5):801–804.

57. Tuntasuvan D, Teeraphan A, Phoengpong N, Jitnupong W, Lungka G. Comparison of serum chemistry values and serum mineral values between captive and free-raging elephants in Thailand [Internet]. International workshop on the domesticated Asian Elephant, 5 to 10 February 2001. 2002. 213-218 p. Available from: http://archive.defra.gov.uk/wildlife-pets/zoos/documents/elephant-forum-1007.pdf

58. Videan EN, Fritz J, Murphy J. Effects of aging on hematology and serum clinical chemistry in chimpanzees (Pan troglodytes). Am J Primatol. 2008;70(4):327–

338.

59. White* PT, Brown IRF. Haematological studies on wild African elephants Loxodonta africana. J Zool. 1978;185(4):491–503.

60. Zhou X, Hansson GK. Effect of Sex and Age on Serum Biochemical Reference Ranges in C57BL / 6J Mice. Comp Med. 2004;54(2):176–178.

Table 1. Body condition score (BCS) and blood pressure values with reference intervals (RI) for semi-captive Asian elephants in Myanmar. A lowercase letter a indicates statistically significant difference between males and females.

						95% Confidence Interval		
Parameter	sex	n	Mean±SD	Median	Range	RI	Lower	Upper
BCS	All	748	3.4±0.6	3.0	2.0-5.0	2-4	2-2	4-4
	Male	281	3.2±0.5	3.0	2.0-4.0	2-4[a]	2-3	4-4
	Female	467	3.4±0.6	3.0	2.0-5.0	3-4[a]	2-2	4-4
Systolic Pressure (mmHg)	All	242	134±16	133	93-172	99-166	93-106	162-170
	Male	105	134±17	133	93-172	100-168	95-104	163-173
	Female	137	134±15	133	93-170	101-165	93-109	160-170
Diastolic Pressure (mmHg)	All	242	96±15	96	53-137	67-127	63-71	123-135
	Male	109	97±18	96	53-137	62-132	57-66	127-137
	Female	136	96±13	96	63-130	68-121	63-74	117-130

Table 2. Hematology values with reference intervals (RI) for semi-captive Asian elephants in Myanmar. A lowercase letter a indicates statistically significant difference between males and females.

						95% Confidence Interval		
Parameter	Sex	n	Mean±SD	Median	Range	RI	Lower	Upper
Hematocrit (%)	All	765	34±3	34	28-44	29-42	29-29	41-42
	Male	278	34±3	33	28-43	28-41	28-29	41-42
	Female	487	35±3	35	28-44	29-42	29-30	41-43
Hemoglobin (g/dl)	All	603	11.7±1.2	11.6	8.5-17.0	9.5-14.3	9.5-9.9	13.9-14.6
	Male	218	11.5±1.2	11.6	9.2-15.9	9.3-14.3	9.2-9.5	13.9-14.6
	Female	385	11.8±1.1	11.9	9.2-16.0	9.9-14.3	9.9-9.9	13.9-14.6
WBC (10^9/L)	All	749	15.8±3.8	15.4	7.7-29.7	9.5-24.6	9.2-9.9	23.3-26.0
	Male	282	15.8±3.7	15.3	8.0-29.6	9.4-24.5	8.8-10.4	22.7-27.9
	Female	467	15.8±3.9	16.0	7.7-29.7	9.6-25.1	9.0-10.1	23.3-27.1
Lymphocytes (%)	All	662	30±8	30	11-52	16-46	14-16	44-47
	Male	249	30±8	30	11-52	17-46	12-18	44-48
	Female	413	30±8	30	11-50	15-46	14-16	44-47
Monocytes (%)	All	667	38±9	38	15-61	21-55	19-23	54-56
	Male	250	38±8	38	18-56	23-55	21-55	52-56
	Female	418	38±10	39	15-61	20-55	17-22	54-56
Heterophils (%)	All	660	27±8	27	11-52	14-44	13-15	43-45
	Male	247	28±8	28	12-52	14-44	12-16	42-51
	Female	413	27±8	27	11-51	14-44	12-16	43-47
Eosinophils (%)	All	667	4±3	4	0-14	0-11	0-0	10-13
	Male	250	4±3	3	0-14	0-11	0-0	9-11
	Female	416	4±3	4	0-14	0-12	0-0	10-13

Table 3. Serum chemistry values with reference intervals (RI) for semi-captive Asian elephants in Myanmar. A lowercase letter a indicates statistically significant difference between males and females.

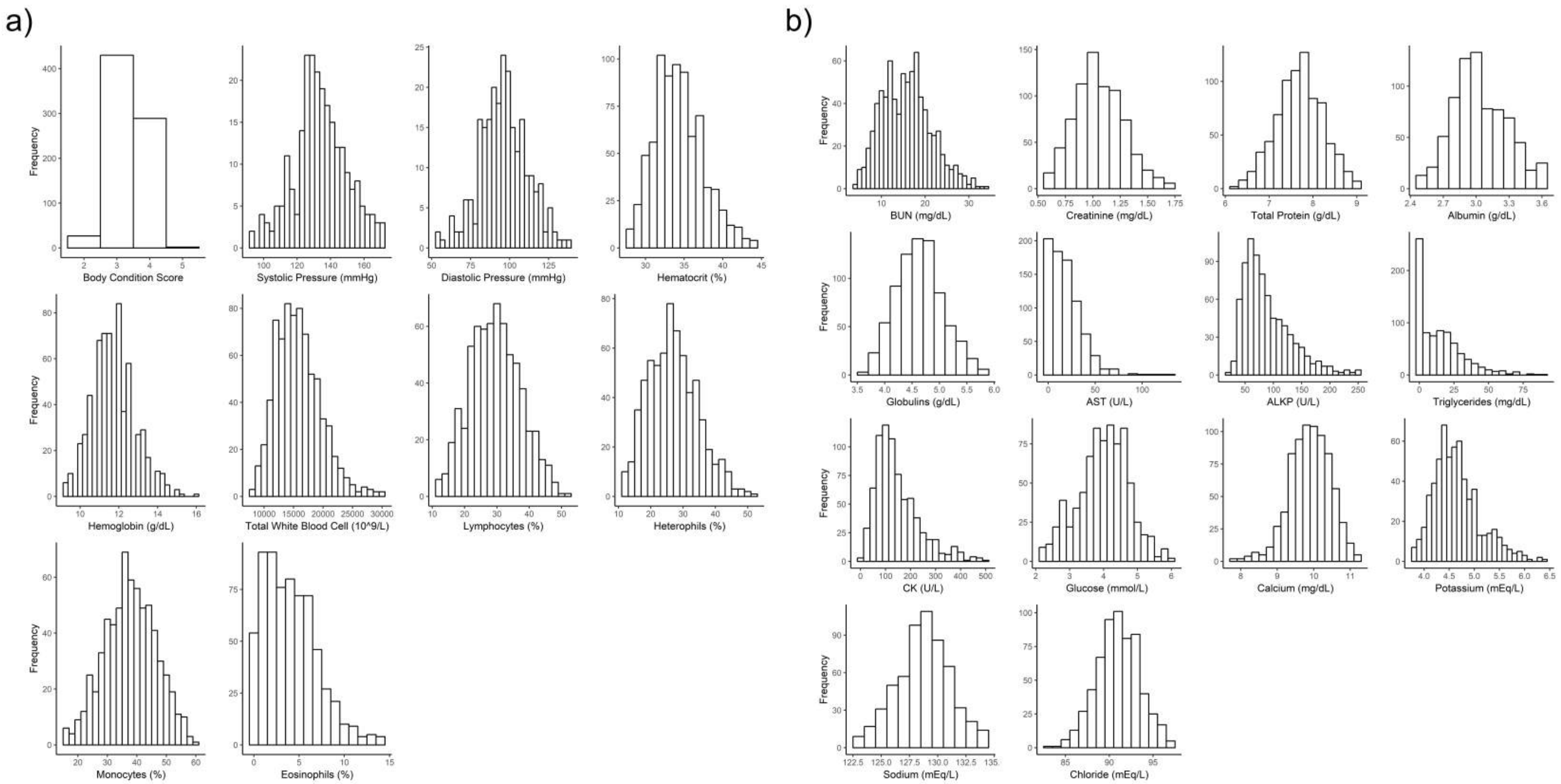
							95% Confidence Interval	
Parameter	Sex	No.	Mean±SD	Median	Range	RI	Lower	Upper
BUN (mg/dL)	All	784	16±6	16	4-34	6-28	6-7	27-29
	Male	290	16±6	16	5-34	5-28	5-7	27-29
	Female	494	16±5	16	4-33	7-28	6-7	26-30
Creatinine (mg/dL)	All	761	1.1±0.2	1.1	0.6-1.7	0.7-1.5	0.6-0.7	1.5-1.6
	Male	284	1.1±0.2	1.1	0.6-1.7	0.7-1.6[a]	0.6-0.7	1.5-1.7
	Female	477	1.0±0.2	1.0	0.6-1.7	0.7-1.5[a]	0.6-0.7	1.4-1.6
Total Protein (g/dL)	All	772	7.7±0.5	7.8	6.2-9.0	6.7-8.8	6.6-6.8	8.7-8.9
	Male	287	7.6±0.6	7.5	6.2-9.0	6.6-8.7	6.4-6.7	8.6-8.9
	Female	485	7.8±0.5	7.8	6.2-9.0	6.8-8.9	6.6-6.9	8.7-8.9
Albumin (g/dL)	All	746	3.0±0.2	3.0	2.5-3.6	2.6-3.6	2.5-2.6	3.5-3.6
	Male	279	3.0±0.2	3.0	2.5-3.6	2.6-3.6	2.5-2.6	3.4-3.6
	Female	467	3.1±0.3	3.0	2.5-3.6	2.6-3.6	2.5-2.6	3.6-3.6
Globulins (g/dL)	All	771	4.7±0.4	4.7	3.6-5.8	3.9-5.6	3.9-4.0	5.5-5.6
	Male	289	4.6±0.4	4.5	3.6-5.8	3.8-5.6	3.8-3.9	5.4-5.7
	Female	482	4.8±0.4	4.8	3.7-5.8	4.0-5.6	3.9-4.1	5.5-5.6
AST (U/L)	All	782	19±17	16	0-128	0-58	0-0	53-69
	Male	289	18±18	16	0-117	0-55	0-0	50-105
	Female	493	19±17	16	0-128	0-62	0-0	55-71
ALKP (U/L)	All	779	90±42	78	20-249	37-199	35-39	187-212
	Male	289	102±48	92	27-249	38-229[a]	36-40	199-247
	Female	490	83±37	73	20-247	36-185[a]	31-39	171-194
Triglycerides (mg/dL)	All	790	15±16	11	0-88	0-58	0-0	52-62
	Male	292	12±13	8	0-70	0-44[a]	0-0	39-50
	Female	498	17±17	13	0-88	0-61[a]	0-0	57-68
CK (U/L)	All	761	147±89	123	11-491	31-385	26-33	369-407
	Male	282	146±90	121	19-486	29-386	22-41	335-455
	Female	479	147±88	124	11-491	38-385	26-35	367-408

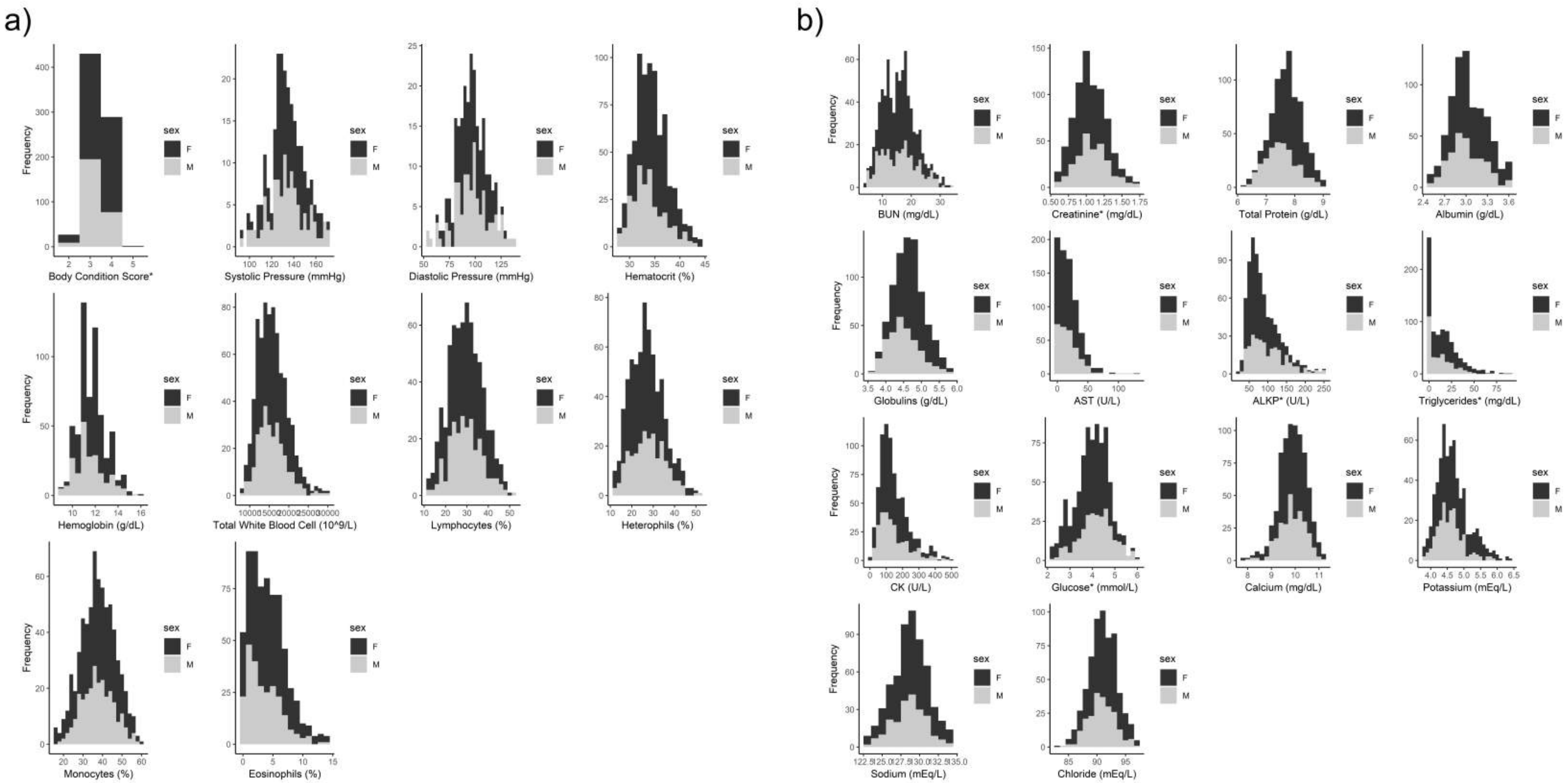
Glucose (mmol/L)	All	778	4.0±0.7	4.1	2.2-6.0	2.5-5.5	2.4-2.6	5.4-5.6
	Male	287	4.2±0.8	4.2	2.3-6.0	2.6-5.8[a]	2.5-2.8	5.5-5.9
	Female	491	4.0±0.7	4.1	2.2-6.0	2.4-5.3[a]	2.3-2.6	5.0-5.4
Calcium (mg/dL)	All	767	9.9±0.6	10.0	7.8-11.2	8.6-11.0	8.4-8.9	10.9-11.1
	Male	283	9.9±0.5	9.9	8.0-11.2	8.9-10.8	8.2-9.0	10.8-11.1
	Female	484	10.0±0.6	10.0	7.8-12.2	8.6-11.0	8.4-8.9	10.9-11.1
Potassium (mEq/L)	All	601	4.7±0.5	4.6	3.8-6.4	3.9-5.8	3.9-4.0	5.6-5.9
	Male	218	4.6±0.4	4.6	3.8-5.9	3.9-5.6	3.4-4.0	5.4-5.8
	Female	383	4.7±0.5	4.6	3.8-6.4	3.9-5.9	3.8-4.0	5.7-6.0
Sodium (mEq/L)	All	589	129±2	129	123-134	124-133	123-124	133-134
	Male	213	129±2	129	123-134	124-133	123-125	132-134
	Female	376	129±2	129	123-134	124-134	123-124	133-134
Chloride (mEq/L)	All	606	91±2	91	83-97	86-96	86-87	95-96
	Male	213	91±3	91	84-97	86-96	84-87	94-96
	Female	376	91±3	91	83-97	86-96	85-87	95-96

Figure Captions

Figure 1 - Frequency distributions for health parameters, including clinical examination/ hematology (a) and serum chemistry (b) parameters, in a population of semi-captive Asian elephants in Myanmar.

Figure 2 – Frequency distributions for health parameters, including clinical examination/hematology (a) and serum chemistry (b) parameters, for male and female timber elephants.


a)
b)
Frequency
Body Condition Score
Systolic Pressure (mmHg)
Diastolic Pressure (mmHg)
Hematocrit (%)
Hemoglobin (g/dL)
Total White Blood Cell (10^9/L)
Lymphocytes (%)
Heterophils (%)
Monocytes (%)
Eosinophils (%)
BUN (mg/dL)
Creatinine (mg/dL)
Total Protein (g/dL)
Albumin (g/dL)
Globulins (g/dL)
AST (U/L)
ALKP (U/L)
Triglycerides (mg/dL)
CK (U/L)
Glucose (mmol/L)
Calcium (mg/dL)
Potassium (mEq/L)
Sodium (mEq/L)
Chloride (mEq/L)


a)
b)
Frequency
sex
F
M
Body Condition Score*
Systolic Pressure (mmHg)
Diastolic Pressure (mmHg)
Hematocrit (%)
Hemoglobin (g/dL)
Total White Blood Cell (10^9/L)
Lymphocytes (%)
Heterophils (%)
Monocytes (%)
Eosinophils (%)
BUN (mg/dL)
Creatinine* (mg/dL)
Total Protein (g/dL)
Albumin (g/dL)
Globulins (g/dL)
AST (U/L)
ALKP* (U/L)
Triglycerides* (mg/dL)
CK (U/L)
Glucose* (mmol/L)
Calcium (mg/dL)
Potassium (mEq/L)
Sodium (mEq/L)
Chloride (mEq/L)