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Study on Some Clinical Important Physiological and Hematological Reference Values for Indigenous Apparently Healthy Donkeys in Around Asella Town, Oromia Regional State, Ethiopia

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Abstract
In the tropical regions Donkeys are exposed to the effects of various environmental factors that alter their physiological and hematological values, which is particularly significant in working Donkeys. However, very little has been done to establish hematological and physiological reference values of local breeds of donkeys in Ethiopia. This study was aimed to determine hematological and physiological reference values for apparently healthy indigenous donkeys breeds in order to form a basis for clinical interpretation in and around Asella town of Oromia regional state, Ethiopia. The study was conducted from January to July 2021 by taking into account vital physiological parameters like body temperature, heart rate, and respiration rate from a total of 32 study animals. All appropriate records for physiological and blood samples for hematological analysis were collected from 32 donkeys. There was a statistically significant difference ($P<0.05$) in the physiological heart and respiratory rates against the age of the study donkeys. However, there was no statistically significant ($P>0.05$) difference in all hematological values of donkeys among sex groups. The mean values of body temperature obtained in this study were: young (37.12±.6.05 ºC) and adult (37.55±0.58 ºC) donkeys. Similarly, for female and male donkeys were 37.55±0.58 and 37.12 ±6.05ºC, respectively. The mean values of heart rate for young and adult donkeys were 46.93±5.31 and 38±3.98, respectively. The mean values of heart rate for female and male donkeys were 58.08±11.2 and 52.46±7.32, consecutively. The present study result should serve as a reference purpose after proper validation in the study animals and further studies in a various agro-climatic zone of the country, Ethiopia.
Keywords: Age, Asella, donkeys, hematological, parameters, physiological, sex.
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1. INTRODUCTION

Equine's livings in the tropical region are subject to the effects of various environmental factors, of which disease conditions are rampant with a significant impact on the performance of animals.[10] Equids have spent a long time being used by man even though little attempts have been made to study their different aspects. They are the most important draught animals playing key roles in the agricultural sector in the developing countries in the form of pack transportation, carting, threshing, farm cultivation, riding, and milk and meat production for humans.[35]

Based on the report of the central statistical authority of Ethiopia, there are 3.77 million donkeys in Ethiopia.[8] Ethiopia possesses the highest equine population which is probably in the highest density per square km in the world and donkeys alone account for 55% of the national equine population which is equivalent to 11.4% of the world and 33.4% of the African donkey population.[2]

Nowadays, many countries have established the normal reference values of clinical and hematological parameters for their local animal species.[23] The diagnosis of the disease is mainly dependent upon the deviations from the normal ranges of physiological values. However, in the absence of established reference values for any hematological parameters, one can be exposed to misinterpretations in diagnosis and treating different disease conditions.

In the present study areas have been no works attempted to determine the normal physiological and hematological reference profiles of local breeds of donkeys except two works done on donkeys breeds in Addis Ababa by Lemma and Moges (2009)[23] and in other study area of Ethiopia by Feseha (1994)[13]. Thus, the aims of the present study were to determine physiological and hematological reference values, and to assess the effect of sex as well as age factors on hematological and physiological parameters among indigenous apparently

healthy donkeys in and around Asella town, Oromia Regional State, Ethiopia in order to put a base line for clinical interpretation.

2. MATERIALS AND METHODS

2.1. Study area description

The study was carried out from January to July 2021 in and around the Asella area of Oromia Regional State, Ethiopia. The climatic condition of the town is "woynadega" and the town is located at an altitude of 2350-2400 meters above sea level. The day and night temperatures of the area ranges from 10-25^{0}C and 10-20^{0}C, respectively. The area has a bimodal rainfall occurring from March to April (short rainy season) and from July to October (long rainy season) with an annual rainfall of 3500- 3550 mm and relative humidity of 43-60%.[19]

2. 2. Study animals

All study animals were reared in free to graze on pasture, with rare provision of other supplements like straw and grains mostly at afternoon, clinically healthy, and free of internal and external parasites. They were treated for endoparasite control twice a year and their health status was regularly monitored by veterinarians in the study areas. Coprological examinations were carried out using the flotation method and all the animals had negative results. The criteria considered being animals apparently healthy, the following conditions were included: healthy animal is alert and aware of its surroundings, should stand on all of its feet and separation of an animal from its group. Animals with severe back sores were also excluded from the study population. Sick animals and animals with body condition scores less than 2 were also excluded. All these criteria are taken into consideration while sampling. Clinically important physiological parameters like rectal temperature, respiratory rates, heart rates, pulse rates, capillary refill time and gut sound were recorded from all study animals.

All study indigenous apparently healthy donkeys in and around Asella district consisting of young (<3 years of age) and adult (3-8 years of age) groups that were randomly selected from households. Age of the study animals was determined by dentition.[10] Blood samples were collected from the study donkeys from jugular vein into 5 ml EDTA coated tubes for hematological analysis.

2.3. Study design

A cross-sectional study was conducted to obtain baseline information concerning the normal physiological and hematological reference parameters in local apparently healthy donkeys in and around Asella town, Oromia, Ethiopia. Data were entered into Microsoft excel spread sheet and analyzed by SPSS V 15. Descriptive statistics was applied to determine the mean (±sd) and range.

2.4. Clinical Examination

Clinically important physiological parameters like rectal body temperature (ºC), respiratory rate (breath/min.) and heart rate (rate/min.) were recorded one by one from all 23 selected donkeys approximately at the same time of the day at 7:00-9:00am. Rectal body temperature was taken by using a digital thermometer and respiratory rate from the tracheal region using a stethoscope. Heart rate was also taken from the left side of the thorax by using a stethoscope. Gut sounds were measured using stethoscope. Pulse rate was taken from the mandibular artery. Capillary refill time was measured from the gum.

2.5. Haematological analysis

From each study animals were obtained 5ml blood samples by jugular vein puncture (Vena jugularis external) in the vacuum tubes with ethylenediaminetetraacetic acid (EDTA) coated tube for hematological analysis including total erythrocyte count (TEC) and total leukocyte count (TLC). Immediately after blood collection, the capped tubes containing the anticoagulant and the blood Samples were inverted gently about 4-5 times to mix. All collected blood samples were transported to the regional laboratory using icebox with icepack.

A hemoglobinometer method was used to determine Hb concentration (g/L) whereas total Red blood cell (TRBC) and total white blood cell (TWBC) count were performed manually using a hemocytometer according to Campbell (995) after the blood samples were mixed by using an automated KJMR-IV blood mixer. Packed cell volume percent (PCV) was determined by a standard manual method using microhematocrit capillary tubes and centrifuged at 2500 rpm for 5 min from Schalm haematology [34] (Weiss D.J., Wardrop K.J., 2010), whereas mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined from TRBC, PCV and Hb according to Ritchie et al. (1994)[27], respectively.

2.6. Statistical analysis

Data were entered into a Microsoft Excel spreadsheet and analyzed by SPSS V15. Descriptive statistics were

used to determine the mean (+SD) and range. The results were considered statistically significant when $P < 0.05$.

3. RESULTS

The results of hematological and physiological parameters in apparently healthy donkeys were statistically processed through a t-test. The mean value and the standard deviation for hematological parameters (Red blood cell, white blood cell, packed cell volume, hemoglobin) and physiological parameters (respiratory rate, breath/minute; heart rate, beat/minute and rectal body temperature, ^{0}c) are presented in Table 1 and 2.

All the hematological and physiological parameters of donkeys between sex and age were found in the normal range. However, there was some significant difference in the physiological parameters between ages and there was a minor difference in mean values between age and sex. The significant difference is found in rectal temperature by age were ($P<0.05$) ($P= 0.0300$).

Table 1: Results of hematological and physiological parameters of study donkeys to compare between sex (male and female).

Parameters	Mean±sd of Female(n=15)	Range of exotic female breed	Mean±sd of male(17)	Range of exotic male breed
Temperature(°C)	37.12±0.58	37 - 38.7	37.55±.6.05	36.3-38.2
HR (b/minute)	52.46±7.32	42-71	58.08±11.2	41-68
RR (b/minute)	40.41±9.86	22-54	42±9.83	31-61
RBC (10^6/µl)	8.27±9.41	6.4-9.41	7.72±7.32	6.4-8.91
WBC (10^3/ µl)	8.90±1.45	6.10-10.00	8.41±1.19	6.45-10.00
PCV (%)	41.43±4.40	32-47	41.98±5.22	34-46.1
Hb (g/dl)	16.53±2.26	11.5-19	16.05±2.52	11.5-18.7
MCV (fl)	50.33±5.77	35-56	53.45±7.29	46-72
MCH (pg)	20.42±3.15	16-26	21.09±3.94	13-25
MCHC (g/dl)	39.5±7.42	31-54	38.55±8.84	26-50

Table 2: Results of hematological and physiological parameters of study donkeys to compare between age (adult and young)

Parameters	Mean±sd of adult (n=18)	Range of exotic adult breed	Mean±sd of young (n=14)	Range of exotic young breed
Temperature (°c)	36.88±0.34	36.3-37.2	37.51±0.62	36.4-38.7
HR (b/minute)	49.68±5.38	42-58	57.41±10.27	41-71
RR (b/minute)	35.27±3.31	31-41	43.29±10.31	22-61
RBC (10^6/µl)	8.21±6.86	6.9-8.91	8.11±6.86	6.4-9.41
WBC (10^3/ µl)	2.58±3.48	1.08-9.50	2.42±2.97	1.00-9.50
PCV (%)	41.87±3.99	34-45	41.72±5.12	32-47
Hb (g/dl)	16.35±2.26	11.5-18.7	16.26±2.89	11.5-19
MCV (fl)	52.12±7.61	48-53	51±2.097	35-72
MCH (pg)	20.82±3.11	13-25	20.5±4.72	16-26
MCHC (g/dl)	38.5±8.26	26-50	39.24±8.12	28-54

4. DISCUSSION

Hematological tests in donkeys help in the clinical diagnosis of systemic, infectious diseases, and some parasitic diseases. It can also provide important information on the treatment response and metabolic status of horses.[9]

The present study result indicated that physiological parameters of donkeys were found within the reference ranges which was different from the result previous reported by Fielding et al. (199)[14]; 8French and Patrick (1995)[15]; Manyahilishal et al. (2011)[24]; Simenew *et al*. (2011)[29]. The difference could be due to breed, climatic factors, season, management, sample size, and different methods used and this strongly signifies determination of these physiological parameters is critically important for each local species of animals in every country. Hence, the results of this study are compared with available research findings on a donkey in other countries. In the present study the higher body temperature was observed in young study animals while compared to adult animals in line with the result reported by French and Patrick (1995)[15].

This high body temperature in young donkeys was attributed to their high metabolic rate (Fielding and Krause, 1998).[14] The lower body temperature values in older animals and slightly higher mean body temperature in females that agreed with study result reported by Kelly (1977)[20]. The mean pulse rate value for females in this study was higher compared to males. This result is in agreement with the result reported by Canacoo *et al.,* (1991)[7] and the observations made by Kelly (1997)[20] on most domestic animals. As reported by French and Patrick (1995)[15], in this study significantly higher respiratory rate was observed in young age

groups compared to adult donkeys.

The positive correlation observed among temperature, heart rate, pulse rate, and respiration rate indicated that as one of these parameters increases the others are also expected to rise. It has been shown that the overriding environmental factor affecting the body functions of domestic animals is temperature. In the present study, the mean values of RR, HR, and Temperature of donkeys were in agreement with the study reported by Simenew *et al*. (2011)[29]. The physiologic responses of the animal during exposure to environmental stress have been determined by variations in body temperature, respiratory rate, and heart rate. Changes in body temperature, respiratory rate, and heart rate are used as indices of meteorological stress, whereas the absence of any change serves as an index of tolerance. [5]

In the present study, physiological values were significantly influenced by age in donkeys. According to Gul *et al*. (2007)[18] reports, the physiological values could not influenced by age and sex differences in donkeys but variations exist between species in contrast with the present study result. These were may be due to environmental, genetic makeup, season, management, sample size factors. Rectal temperature (T^oc) of donkeys between ages were P=0.0300.

In a hot humid climate, the ability of the air to absorb additional moisture is limited, and inadequate cooling may result in heat stress. High body temperature initiates compensatory and adaptive mechanisms to re-establish body homeostasis. Thus, the physiologic responses of the animal during exposure to environmental stress have been determined by variations in body temperature, respiratory rate, and heart rate. Changes in body temperature, respiratory rate, and heart rate are used as indices of meteorological stress, whereas the absence of any change serves as an index of tolerance.[5]

Hematology has been widely used to provide information about the disease status, performance problems, and fitness in equine species. Age, sex, and breed as well as physical exercise may affect the results of hematological values. In the present study, there were no significant difference (P>0.05) between sex and age in mean values of heamatological parameters in donkeys. So, this study were in agreement with study reported by Etana *et al.* (2011)[12].

Despite the prolonged use of hematology in equine medicine, interpretation can be a challenge in some cases because it can be influenced by a large number of factors. Hematologic parameters may vary according to breed, sex, age, reproductive status, physical form, training level, feeding, animal handling, blood sampling, degree of excitation, and state of health (Kramer, 2000).[21]

In the present study, the mean value of the number of red blood cells (RBC), the packed cell volume (PCV), MCV, MCH, WBC, MCHC, and hemoglobin (Hb) were found within the normal range for all female and male groups with no significant difference were observed for these parameters that were in line with results reported by Etana *et al.* (2011)[12].

The present study on hematological values of donkeys were in close agreement with the observation of Archer and Jeffcott loc. cit (1977)[4] and were not significant influenced by sex. Males have higher values of temperature, heart rate, respiratory rate, packed cell volume, mean corpuscular hemoglobin, and mean corpuscular volume than in female, while in females have higher Hb, WBC, RBC, and MCHC values than male in contrast with the results reported by Satue *et al*. (2009)[28]. The findings of the current study showed that the mean blood cell volume (MCV) and mean corpuscular Hb concentration (MCHC) were found within the normal range for all study groups in the present study areas. There was no statistically significant difference (P>0.05) concerning sex in donkeys.

Where variation is observed between the values of the current study and earlier reports on donkeys, it could be as a result of bio-variations in breeds and environmental conditions (Aluja *et al*., 2001; Tesfaye *et al*., 2014).[3, 32] Stress imposed by seasonal weather changes which can influence variations in values of normal respiratory rate, pulse rate and rectal temperature of donkeys (Ayo *et al*., 2008). [5]

The mean values for WBC was higher in adult than in young donkeys that was also in agreement with results reported by French and Patrick (1995)[15] and Terkawi *et al.* (2002)[31]. In the present study, adult donkeys had higher values of RBC, WBC, PCV, Hb, MCV, and MCH values than young ones but their values of heart rate (HR), temperature (T^0), respiratory rate (RR), and MCHC were less than that of young donkeys. Young donkeys had higher values of respiratory rate (RR), heart rate (HR), temperature (T^0), and MCHC. This may be due to environmental factors, management factors, and genetic makeup. In the present study, all hematological parameters were found within the recommended reference ranges for donkeys that in agreement with the result reported by Weiss and Wardrop (2010) [34].

Values of PCV and Hb are used in the assessment of the metabolic profile of animals (Zakari *et al*., 2014).[37] The mean value of PCV detected in the present study was different from the results reported by Caldini *et al*. (2005)[6]. In the present study, the mean values of MCH of adult and young donkeys were similar with the previous study reported by Caldini *et al*. (2005)[6]. The mean value of MCHC in the present study was higher (39.04±7.95) than the result (31.5-35.2 g/dl) reported by Stanisic et al. (2015) [30] on donkeys. In the present study MCHC values were higher but lower Hb value which may be due to season and climate conditions of

sampled donkeys.

Hematological values for trend donkeys with age groups showed that adults had higher RBC, Hb, MCV, MCH, PCV value than young donkeys. These findings were in agreement with Etana *et al.* (2011)[12] and Canacoo *et al.* (1991)[7] but for the values of MCHC which were high in young. Terkawi *et al.,* (2002)[31] also reported a higher MCV value in adults that agreed with the present study.

Statistically significant differences between males and females were not found in the present studies that in line with the result reported by Yakubu and Chafe (2008)[36]; Folch *et al.*(1997)[16]; French and Patrick (1995)[15]; Dinev and Khubenov (1986)[11].

Zinkl et al. (1995)[38] reported that females had higher MCHC, leukocyte and neutrophils count than males but also Nayeri (1978)[26] reported that females having higher Hb, MCV, MCH, MCHC, and PCV. In the present study the mean value of MCHC (39.04±7.95) obtained was different from the previously results (36.3 g/dl, 35.93 g/dl, and 36.8 g/dl) reported by Laus *et al*. (2015)[22], Mot *et al.* (2010) [25] and Simenew *et al.* (2011) [29], respectively for donkey breeds. This may be due to breed, genetic makeup, age, sex, feeding, season, and climate. According to Caldini *et al.,* (2005)[6] reported higher MCHC values for Ragusana donkeys than Sicily (40.6g/dl). This report had a slight difference when compared to the present studies. In the present study the mean value of MCHC in donkeys (31.5 – 35.2 g/dl) was higher than the result reported by Al-Busadah and Homeida (2005)[1], Etana *et al*., (2011) [12], Folch *et al*. (1997)[16], Lemma and Moges (2009)[23], Stanisic *et al. (*2015)[30], Vucicevic *et al. (*2011)[33]. This difference may be due to season, genetic, breed, sex, environmental condition, feeding and management factors.

Values of PCV and Hb are used in the assessment of the metabolic profile of animals (Zakari *et al*., 2014).[37] The mean value of PCV for females and males donkeys was registered in the current study 41.43±4.40 and 41.98±5.22, respectively. Caldini *et al*. (2005)[6] research result on Ragusana donkeys from Sicily (Italy) (28.5%) was also higher than that reported in the previous studies on donkeys by Folch *et al*.(1997)[16]; Laus *et al.*(2015)[22]; Stanisic *et al.*(2015)[30]; Vucicevic *et al.* (2011)[33].

In the present studies the mean value of Hb obtained (16.3±2.34) was different from Etana *et al*. (2011) [12] research reported on local breed donkeys of Ethiopia (10.84g/dl) and Vucicevic *et al.* (2011)[33] research reported on domestic Balkan donkey (10.43g/dl). On the other hand, in Gul *et al*. (2007)[18] research report on Pakistan donkeys stated that the mean value of Hb was lower (9.01g/dl) than with present study value recorded. In the current study, the mean value of Hb (16.3±2.34g/dl) was higher than the value that reported by Al-Busadah and Homeida (2005)[1], Caldini *et al.* (2005)[6], Laus *et al. (*2015)[22], Mot *et al.* (2010)[25], Simenew *et al.* (2011)[29], Stanisic *et al.* (2015)[30]. Results of the Hb value obtained in the present research support the hypothesis of Zakari *et al*. (2014)[37] that values of Hb were lower in the summer. This difference with current studies may be due to season, sex, age, agro ecology, and management factors.

The mean values of WBC obtained in the present study were different from the result (9.83 $x10^9$/L) reported by Caldini *et al.* (2005)[6] on Ragusana donkeys and by Gul *et al.* (2007)[18] result (9.75 $x10^9$/L) reported on Pakistan breeds donkey. Also, the mean value of WBC obtained in the present study was lower than from the value reported by Garba *et al*. (2015)[17] and Mot *et al.* (2010)[25] 7.2 $x10^9$/L and 6.37 $x10^9$/L, respectively. This difference may be due to geographical factors, environmental factors, management factors, breed, sex, and genetic makeup.

5. CONCLUSION AND RECOMMENDATIONS

Donkeys have been neglected despite their prominent roles in both the rural and urban societies of the country and hence research on donkeys has been lagged far behind other domestic species. Reference values of apparently healthy animals serve as a guide to clinicians in evaluating clinical findings. In this study, the mean value of heart rate, respiratory rate, and rectal temperature in young donkeys had higher than adult donkeys. But adult donkeys had higher mean values of RBC and WBC than young donkeys. Female donkeys had higher WBC, RBC, and mean corpuscular hemoglobin concentration than male donkeys. While male donkeys had higher heart rate, mean values of respiratory rate, mean corpuscular volume, and mean corpuscular hemoglobin than female donkeys.

In this study, hematological and physiological values were determined for local working donkeys of Asella breed, Ethiopia and therefore, considerable differences in these values attributed to variation in age and sex should be noted during the physical clinical examination which will certainly help a clinician to make the correct diagnosis of a condition. The slight differences found between our results and those reported in previous works confirm the need for further studies to investigate the normal reference values of hematological and physiological values in Asella donkeys breed. In light of the above conclusion the following recommendations are forwarded:

- Further studies of hematological and physiological values in apparently healthy donkeys in different areas of Ethiopia should be required.
- Researches on local donkeys that focuses on health and welfare should be strengthened.

- The study on donkeys should have continued to investigate factors affecting their hematological and physiological parameters.

Conflict of Interests
There were no conflict of interests in this research.

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