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Determination of Reference Hematological and Serum-Biochemical Values for Working Donkeys of Ethiopia

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Abstract: This study was undertaken with the aim of determining reference hematological and serobiochemical values for working Ethiopian donkeys in four districts of Oromia regional state. The study was conducted by taking blood and serum samples from a total of 130 apparently healthy donkeys which were analyzed by using automated hematology analyzer and photometer 5010, respectively. There were significant difference in RBC ($p<0.001$), PCV ($p<0.04$), MCV ($p<0.001$) and MCHC ($p<0.001$) among the different age groups. The differences in mean values for PCV, Hb, MCV, MCHC, MCH, Hb, total WBC, eosinophils, monocytes and Platelets between sexes were not statistically significant ($p>0.05$). In the serobiochemical studies significant difference was seen only in the mean creatinine ($p<0.003$) values among the different age groups. Therefore, the variations in hematological and serum biochemical values ascribed to the effects of age and sex should be taken into consideration in interpreting physiological hematological and serum biochemical parameters in working donkeys.

Key words: Apparently healthy, donkey, hematological, serum-biochemical, serum, Ethiopia

INTRODUCTION

Ethiopia has the large donkey population in Africa which accounts to 11.4% of the world's and 33.4% of the African's donkey population (Getachew, 1993). According to the present regional classification of Ethiopia, 97% of the donkeys are found in three regions: 44% in Oromia, 34% in Amhara and 19% in Tigray regional states (Feseha, 1998). Donkeys are one of the most important domestic animals most intimately associated with man. They contribute a lot through their involvement in different social and economic sectors.

In Ethiopia donkeys have been used as beast of burden for long time and still render their valuable services, mostly as pack animals throughout the country in general and in areas where modern means of transportation are absent, unaffordable or inaccessible in particular (Getachew *et al*., 2002). Despite their prominent role in agricultural lead policy of the country, the knowledge based on physiology, nutritional requirements, health problems and care and management systems are limited and rarely available in the literature except the attempts made by The Donkey Sanctuary since its inception (Feseha, 1998) and hence donkeys have low status in many of the regions where they are now found (Pearson and Merritt, 1991; Getachew, 1993). Disease diagnosis in animals is largely dependent on the physical clinical examinations and laboratory test results expected to reflect biological variations. This in turn requires understanding of reference hematological and serobiochemical profiles of an apparently normal animal whose measurement provides invaluable information concerning the health status of an animal in clinical studies.

However, with the exception of the research by Feseha (1994) on some blood parameters there has been no detailed investigation on the reference hematological and serum biochemical parameters of working donkeys in Ethiopia. Therefore, this study was designed to determine reference hematological and serobiochemical profiles of clinical importance for local working donkeys.

MATERIALS AND METHODS

Study area: The study was conducted in 2005 at four districts of Oromia regional state of Ethiopia where the Donkey Health and Welfare Project has been operating for more than a decade namely:

Ada district: It is located at 8°7'N latitude and 39°E. The average altitude is 1900 m above sea level and mean annual rainfall is 115.6 mm. Mean annual maximum and

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minimum temperatures are 30.5 and 8.5°C, respectively with a relative humidity of 61.3% and donkey population was 48,366.

Akaki district: The altitude ranges from 1700-2100 m above sea level and annual rainfall ranges from 100-1300 mm. The average daily maximum and minimum temperatures are 20 and 18°C, respectively and has 16,035 donkey population.

Dugda Bora district: The altitude ranges from 1600-2020 m above sea level and the average annual rainfall is 750 mm. Mean daily maximum and minimum temperatures were 28 and 22°C, respectively and has 12,900 donkey population.

Berhe district: The altitude ranges from 2300-300 m above sea level and mean annual rainfall is 300 mm. The average daily minimum and maximum temperatures were 15 and 28°C, respectively and has 24,395 donkey population.

Study animals and sampling methods: Apparently healthy and working donkeys were the sampling units for this study and all were kept under semi-intensive farming system that means free to graze on pasture and rarely provided with other feed supplements mainly straw and coarse grain usually after strenuous work. Donkeys within the age range of 4 months to 20 years as determined by dentition following description given by the International Donkeys Protection Trust (McCarthy, 1986) were included in the study.

Study animals were then grouped into three age categories: young (<2 years), adult (2-15 years) and old (>15 years) according to the local farming system. Moreover, all donkeys in the study had body condition score between 2 and 2.5 in scaling fashion provided by Svendsen (1997). Of the total 263 donkeys sampled randomly from the above four districts, 138 donkeys were selected for the study and of these 88 (63.88%) were male and 50 (36.12%) were female.

These were selected based on normal physical and clinical examination findings (body temperature, pulse rate, respiration rate, gut sound, mucous membrane, dental examination, skin and musculoskeletal conditions) and those donkeys with abnormalities of any type were withdrawn from the study. Donkeys that had passed the first screening (physical clinical examination) were followed for 2 weeks prior to determining physiological parameters.

During this follow-up period, blood samples from ear vein for haemoparasites and feces for parasitic eggs and larvae were analyzed and donkeys were dewormed with ivermectin irrespective of the EPG count and with triclalbendazole when diagnosed positive for trematode. Yet eight more donkeys were excluded at this stage and eventually 133 (50.6%) donkeys were withdrawn and only 130 donkeys were included in the study and these were 84 (65%) male and 46 (35%) female.

Determination of hematological parameters: The sampling time was adjusted for all donkeys throughout the sampling period to be at 9:00 am. Blood samples were collected by venipuncture in EDTA coated vacutainer tubes (4 mL). The blood was shacked gently and immediately by inverting movement two to three times to mix it well with EDTA.

Determination of serobiochemical parameters: Another blood samples were also collected in plain vacutainer tube (7 mL) at the same time with the above activity by just changing the vacutainer tube while fixing the needle inside the jugular vein. The blood in plain vacutainer tube was also handled gently to avoid haemolysis and kept in a slant position at room temperature until serum had been separated. Then the serum was transferred in sterile vials for immediate analysis otherwise it was stored in a fridge at -20°C.

Laboratory analysis: The blood with anticoagulant was analyzed by Automated Hematology Analyzer FX-21N for Total Red Blood Cell Count (TRBC), Total White Blood Cell Count (TWBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Hemoglobin (Hb) and platelet count. Thin smear was also made from each blood sample collected with anticoagulant and stained with Giemsa stain to determine differential leukocyte count on a 200 cell count to increase accuracy.

Serum samples obtained from plain vacutainer tubes were subjected to standard biochemical procedures using Photometer 5010 to determine normal values of clinically important serobiochemicals including GOT (Glutamic oxaloacetic transaminase)/AST (Aspartate amino transferase), GPT (Glutamic pyruvic transaminase)/ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), urea, creatinine, glucose, total bilirubin and total protein.

Date analysis: Data were analyzed using SPSS Version 15 software to determine the mean values of different physiological hematological and clinically important serum biochemical parameters and to assess the presence of significant difference in the above values with regard to factors supposed to cause variation. About 95% Confidence Interval (CI) and 5% absolute precision were used throughout the study.

RESULTS AND DISCUSSION

The mean with its 95% confidence interval, standard deviation and median of the hematological and clinically

Table 1: Reference hematological values (RBC, PCV, Hb and MCHC) of different age and sex groups of working donkey

Age		RBC (*10^6 µL^{-1})			PCV (%)			Hb (g dL^{-1})			MCHC (g dL^{-1})		
(years)	N	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI
<2	45	5.5±0.74	5.8	4.5-8.2	31.4±4.69	31.7	23.0-38.5	10.4±1.41	10.4	7.8-12.7	33.2±1.20	33.1	31.6-35.2
2-15	71	6.0±1.08	5.5	4.1-6.6	32.1±4.12	32.0	24.6-37.5	11.1±3.15	10.7	8.5-12.7	33.7±0.85	33.6	32.5-35.0
>15	14	5.1±0.96	5.4	3.4-6.3	30.4±4.96	30.7	23.0-38.1	11.1±4.92	10.5	8.0-13.5	33.9±1.02	33.9	32.0-35.7
Female	46	5.5±1.05	5.4	3.9-7.5	30.6±4.83	30.3	23.0-38.5	10.6±3.60	10.4	7.8-12.8	33.6±1.04	33.4	31.9-35.5
Male	84	5.7±0.92	5.7	3.9-7.4	32.1±4.26	32.2	24.6-38.4	11.0±2.80	10.7	8.3-12.8	33.5±1.06	33.4	31.8-35.1

Table 2: Reference hematological values (MCH, MCV, platelets and WBC) of different age and sex groups of working donkeys

Age		MCH (pg)			MCV (fl)			Platelets (*10^3 µL^{-1})			WBC (*10^3 µL^{-1})		
years)	N	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI
<2	45	19.0±8.00	17.6	14.9-20.6	52.5±3.71	52.2	46.9-58.9	228.8±88.70	243.0	92-382	14.10±3.82	13.3	8.9-21.0
2-15	71	19.8±1.10	19.7	18.2-22.3	58.3±4.73	58.8	53.7-63.8	192.2±99.30	189.0	42-329	13.10±2.37	13.0	9.8-17.3
>15	14	20.0±1.20	20.8	18.6-22.4	60.3±3.52	60.6	53.3-65.6	220.6±68.70	232.5	76-306	12.40±2.55	12.3	8.2-16.0
Female	46	19.5±5.77	18.8	15.8-22.1	54.9±5.54	54.8	48.2-62.2	286.5±92.49	253.0	92-358	13.83±3.40	13.8	9.5-19.4
Male	84	19.8±4.35	19.6	15.9-22.4	57.5±4.79	58.4	48.9-63.9	309.2±90.05	202.0	47-332	13.02±2.70	12.6	8.7-18.0

Table 3: Reference values of differential leukocyte count

Age	Neutrophils (%)			Lymphocytes (%)			Eosinophils (%)			Monocytes (%)		
years)	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI
<2	44.2±10.46	46.6	20.6-60.4	50.1±10.59	47.2	34.7-71.4	4.3±0.237	4.3	0.91-8.30	1.60±0.96	1.5	0.13-3.3
2-15	46.3±9.090	45.3	33.9-62.4	45.8±10.11	45.2	28.4-59.7	5.1±3.270	5.3	1.45-11.1	1.50±0.97	1.4	1.40-2.9
>15	46.3±7.790	46.3	36.3-56.3	46.4±8.270	47.3	31.8-54.9	5.6±4.570	4.6	1.42-8.50	2.00±3.04	1.5	0.00-4.1
Female	43.05±9.02	43.2	30.1-59.4	49.99±9.86	49.4	33.3-67.4	5.21±3.95	4.8	1.10-10.8	1.78±2.31	1.3	0.00-4.3
Male	46.96±9.34	47.5	34.9-63.9	46.02±10.0	45.2	28.4-59.8	4.97±2.91	4.5	1.10-9.10	1.53±0.96	1.4	0.10-2.9

Table 4: Reference serobiochemical values (AST/GOT, ALT/GPT, ALP, creatinine) of different age and sex groups of apparently healthy local working Ethiopia donkeys

Age	AST/GOT (iµ L^{-1})			ALT/GPT (iµ L^{-1})			ALP (iµ L^{-1})			Creatinine (µmol L^{-1})		
years)	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI
<2	272.5±71.72	254.0	199-398	12.3±5.710	11.00	7.0-21.1	535.9±197.10	533	317-931	129.9±25.15	139.1	95.8-160.7
2-15	303.9±80.56	307.0	175-423	10.2±3.720	9.30	5.0-17.0	441.7±131.30	410	272-605	127.2±24.37	121.4	100.9-178.0
>15	325.4±72.79	333.3	180-461	12.2±2.780	12.60	6.7-17.6	517.3±166.50	513	272-1082	147.5±30.99	150.5	89.3-208.6
Female	286.5±65.87	282.0	190-418	10.29±3.69	10.00	4.0-17.0	471.0±180.05	425	272-931	123.7±23.21	121.7	95.8-160.7
Male	309.2±84.09	307.0	175-459	11.67±4.47	110.00	5.0-19.0	489.0±151.37	495	272-792	137.6±28.08	133.5	97.5-185.6

Table 5: Reference serobiochemical values (urea, glucose, total protein and total bilirubin) of different age and sex groups of apparently healthy local working Ethiopia donkeys

Age (in	Urea (mmol L^{-1})			Glucose (mmole L^{-1})			Total protein (g dL^{-1})			Total bilirubin (mg dL^{-1})		
years)	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI	Mean±SD	Median	95% CI
<2	7.80±1.96	7.9	5.9-10.1	1.70±0.97	1.60	0.36-3.20	8.10±1.39	8	7-10	0.07±0.26	0.0	0.0-1.00
2-15	7.30±1.47	7.3	5.2-9.40	2.10±1.49	2.10	0.46-3.90	9.10±1.29	9	7-11	0.16±0.37	0.0	0.0-1.00
>15	8.40±1.59	8.1	5.8-10.6	1.90±1.02	1.80	0.53-4.20	8.10±1.23	8	6-10	0.60±0.55	1.0	0.0-1.00
Female	7.77±1.42	7.7	5.9-9.40	1.87±1.08	1.65	0.43-3.96	8.84±1.16	9	7-10	0.05±0.21	0.0	0.0-0.05
Male	7.61±1.83	7.5	5.1-10.6	1.96±1.39	1.70	0.38-4.24	8.45±1.50	8	6-11	0.25±0.44	0.0	0.0-1.00

important serum biochemical values of apparently healthy donkeys are shown in Table 1-5 with respect to variation in age (young, adult and old) and sex. The difference in RBC count, MCV and neutrophil count between different sexes were statistically significant ($p<0.05$) (Table 1). However, there were no significant difference ($p>0.05$) in the values of PCV, MCHC, MCH, Hb, total WBC, Eosinophils, Monocytes and platelets counts between male and female (Table 2). Although, it was not statistically significant the values for lymphocytes were higher in female. The value of basophils was insignificant to consider proportionally along with the other leukocytes (Table 3). There was statistically significant difference ($p<0.0001$) in RBC count between all age groups. The value was higher in adult followed by young and older animals, respectively. The value for PCV was significantly different ($p<0.04$) between old and the other two age groups.

This study also indicated that there was statistical significant difference ($p<0.0001$) in MCV value between all age group, MCV being higher in old. There was statistical significant ($p<0.001$) difference between young and old

animals in the MCHC value; the values were higher in older animals. The value of Hb was significantly ($p<0.05$) higher in adult than old animals. The value for WBC was statistically different ($p<0.03$) between old and the other two age groups. The value was lower in older than the other two age groups. The value of eosinophils and monocytes was significantly ($p<0.05$) higher in older than young donkeys. There was no statistically significant difference ($p>0.05$) between sexes in all biochemical values considered in this study except for creatinine.

The value for creatinine was significantly ($p<0.02$) higher in males. The serobiochemical values were not also significantly ($p>0.05$) different among age groups except for total protein which was significantly ($p<0.003$) higher in adults. Unlike other domestic animals researches undertaken on donkey's medicine, biology, physiology, management and socioeconomics are hardly available in Ethiopia except the attempts made by the animal charity group, The Donkey Sanctuary.

Since there is no reliable and comprehensive data on hematological and serobiochemical values of donkeys in Ethiopia, today veterinary clinicians who rarely treat donkeys have been using the physiological values of horses which might result in an invalid comparison and hence the results of this study are compared with available research findings else where in other countries (Table 4). The trend in hematological values with respect to age showed that adult had higher RBC, Hb, MCV, MCHC, PCV and MCH than young. These findings are in agreement with Canacoo *et al.* (1991) except for the values of MCHC and MCH which were reported to be higher in young. However, Folch *et al.* (1997) stated that Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) tend to increase with increasing in age. Terkawi *et al.* (2002) also reported a higher MCV value in adult in agreement with the present study.

In agreement with this study Brown and Cross (1969) and Zinkl *et al.* (1990) found a significantly lower erythrocyte counts in young donkey populations, suggesting that the smaller sized erythrocytes of young donkeys could be attributable to iron deficiency. In this study the value for RBC, PCV, Hb and MCV was higher in males while MCH and MCHC were higher in females. Except for MCV which is higher in females, these findings were exactly the same as the findings of Enio *et al.* (2004). In agreement with the present study Feseha (1994) also reported a higher value of PCV and MCV in males. RBC and Hb were also reported to be higher in males by Terkawi *et al.* (2002). In contrary to the present study MCH was reported to be higher in males by both Feseha (1994) and Terkawi *et al.* (2002). In the present study the value of MCV for males is significantly higher ($p<0.05$).

The mean value for WBC was higher in young than in adult and older donkeys. Similar result was also reported by French and Patrick (1995) and Terkawi *et al.* (2002). The total and differential leukocyte counts and proportions of cell types in healthy donkeys are related to several factors. The most prominent factor is age which is accompanied by a steady decline of WBC number (Zinkl *et al.*, 1990; Folch *et al.*, 1997). The mean value for neutrophils, eosinophils and monocytes were higher in males than in females. Enio *et al.* (2004) also reported similar results except for eosinophils which is higher in females. However, the mean value for lymphocytes was higher in females than in males. This finding is similar to the result reported by Feseha (1994).

The serobiochemical result obtained in this study were all within the normal value set by Zinkl *et al.* (1990) except for the value of ALP which was higher in this study. Although, there was slight difference in the individual serobiochemical result with regard to age and sex there is no significant difference except for the values of creatinine and total protein (Table 5).

The creatinine value for female was significantly ($p<0.05$) higher than males. The total protein value for adult animals was also significantly ($p<0.05$) higher than young. However, there is no significant ($p>0.05$) difference in the values of total protein between young and old animals. A general tendency for increasing plasma protein values with increasing age has been observed in donkeys (Zinkl *el al.*, 1990).

CONCLUSION

Equine particularly donkeys had been totally neglected despite their prominent role in both the rural and urban society of the country and hence research on donkeys has been lagged far behind other domestic species. Reference values of clinically healthy animals serve as a guide to the clinician in evaluating clinical findings. Therefore, researches on donkeys that focuses on health and welfare should be strengthen. In this study adults had significantly high mean values in most hematology and serum biochemical values than young and older donkeys. Females had also significantly higher mean values in most hematological and serum biochemical values.

In this study hematological and serum biochemical values were determined for local working donkeys of Ethiopia and therefore, considerable differences in these values attributed to variation in age and sex should be noted during physical clinical examination which will certainly help a clinician to make correct diagnosis of a condition.

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