

Reference Values of Clinically Important Physiological, Hematological and Serum Biochemical Parameters of Apparently Healthy Working Equids of Ethiopia

¹K. Simenew, ²M. Gezahegne, ²M. Getachew, ³M. Wondyefraw, ²L. Alemayehu and ²I. Eyob

¹Faculty of Agriculture and Rural Development, Dilla University, P.O. Box 419, Dilla, Ethiopia

²Faculty of Veterinary Medicine, AAU, Debre-Zeit

³Faculty of Medicine, AAU, Addis Ababa

Abstract: Equids living in the tropical region are subject to the effects of various environmental factors that alter their physiological and clinical parameters, which is particularly significant in working equines. Very little has been done to establish reference values and there is paucity information in this regard for working horses, donkeys and mules in Ethiopia. The physiological ranges of selected clinical, hematological and serum biochemical values of apparently healthy working equids were studied with the objective of establishing the reference values. Data for clinical parameters were collected from 408 donkeys, 50 horses and 32 mules while the blood samples were obtained from 315 donkeys, 48 horses and 31 mules. Description of the population samples, laboratory methods and statistical analysis were presented allowing comparison and judicious application of these normal values by interested researchers and clinicians. Considering variations in altitude, sex, age, season, environmental and management factors, further studies are essential for detailed record of physiological parameters in the indigenous breeds of working equids.

Key words: Apparently healthy • Clinical • Equids • Hematological • Serum-biochemical

INTRODUCTION

Equids have spent long time being used by man, even though little attempts have been made to study their different aspect. 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, milk and meat production for human [1-4]. Even these days despite the increase in mechanization throughout the world, equids are still well deserving of the name 'beasts of burden'.

Ethiopia has 65% of all African mules and almost 50% of horses [5]. According to the central statistical authority of Ethiopia [6] there are 321, 389 mules, 3.77 million donkeys and 1.45 million horses in Ethiopia. Ethiopia posses the highest equine population which probably is the highest density per square kilometer 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 African donkey population [6-8]. However, the integration of full utilization of equids is constrained by many production factors of which diseases, low plane of

nutrition, poor genetic potential and poor management are the most important [9, 10]. Equines living in the tropical region are subject to the effects of various environmental factors.

Of all the factors disease conditions are rampant with a significant impact on the performance of animals [10, 11]. The hematological and serum biochemical profiles would substantiate the physical clinical examination together with anamnesis to provide excellent basis for correctly diagnosing the diseases, the extent of organ damage and the response of the defense mechanism. These days, many countries have established the normal reference values of clinical, hematological and serum biochemical parameters for their local animal species [12].

The diagnosis of diseases is mainly dependent upon deviations from the normal range of physiological values. However, in the absence of established reference values for any blood parameters, one can be exposed to misinterpretations in diagnosing and treating different disease conditions. Elaborate works by Donkey Sanctuary Working Worldwide, England [13] and some other works in the tropics [14] established physiological,

hematological and biochemical parameters which are extremely important for the diagnostic purpose of equine diseases.

In Ethiopia there have been no works attempted to determine the normal clinical, hematological and serum biochemical profiles of working equids except two works done on donkeys [12, 14]. The objective of the present study was to determine reference normal values of clinically important physiological, hematological and serum biochemical profiles of equids under tropical climatic conditions of Ethiopia.

MATERIALS AND METHODS

Study Areas and Study Population: The study area comprises central Ethiopia about 200 kms radius from the capital city Addis Ababa. Apparently healthy working equids (donkeys, horses and mules) owned by farmers were sampled. All equids were reared in extensive farming system (free to graze on pasture, with rare provision of other supplements like straw and grains mostly after work).

The criteria considered being animals apparently healthy, the following conditions were included. Healthy animal is alert and aware of its surroundings. It should stand on all of its feet. Separation of an animal from its group is often a sign of health problem. 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 sampled animals were de-wormed and clinical parameters and blood samples were taken after two weeks of de-worming to avoid the confounding effects of parasitism on the normal values. Animals showing exceptionally high or low clinical parameters readings were excluded from the study. All these criteria were taken into consideration while sampling. Clinically important physiological parameters recorded include: rectal temperature, respiratory rates, heart rates, pulse rates, capillary refill time and gut sound taken from 490 working equids. Blood samples were taken from 394 (315 donkeys, 48 horses and 31 mules) equids from jugular vein into 10ml plain tubes for serum biochemistry and 7ml EDTA coated tubes for hematology.

Study Design, Statistical Data Analysis and Methodologies: A cross-sectional study has been conducted to obtain baseline information concerning the normal physiological, hematological and serum biochemical parameters in working equids of Ethiopia.

Data were entered into Microsoft excel spread sheet and analyzed by SPSS V 15. Descriptive statistics was applied to determine the mean (\pm sd) and range.

Assessment of Physical Clinical Examination: All clinically important physiological parameters were taken by one individual to avoid personal bias and approximately the same time during the day at 8:00-11:30. Data checklist was prepared on which measured values were recorded. Temperature was taken using digital thermometer. Respiratory rate was taken from the trachea using stethoscope. Heart rate was also taken using stethoscope. Pulse rate was taken from the mandibular artery. Color of mucus membrane was determined through the examination the conjunctiva and the gum. Capillary refill time was measured from the gum. Gut sounds were measured using stethoscope.

Hematological and Serum Biochemical Parameters: Blood samples were drawn from animals at rest, undisturbed or under least excitement. Blood samples were obtained by jugular vein puncture in vacuum tubes without anti-clotting agent for biochemical analyses and with EDTA 10% for hematology. Immediately after blood collection, the capped tubes containing the anticoagulant and the blood samples were inverted gently about ten times to mix. Samples were transported using icebox with icepack. In the laboratory hematological analysis was performed after mixing the blood using an automated KJMR-IV blood mixer. The red blood cell count, packed cell volume, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood cell and platelet counts were determined using an automated analyzer (POCH-100 IV Diff, Sysmex corporation, Kobe, Japan, 2005).

Serum was obtained by centrifugation (2500 rpm, 15 minutes) and stored at -20°C until analyses were performed. The concentrations of total protein, total bilirubin, direct bilirubin, glucose, triglyceride, urea, creatinine and the activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase were also determined using automated systems photometer 5010 (Robert Riele GmbH and coKG, Berlin, Germany, 2002). Serum electrolytes: sodium, potassium, chloride and calcium were analyzed using Roche AVL 9180 Snap pack Electrolyte Analyzer (Roche Diagnostic Corporation 9115 Hague road, USA, 2002). The results were automatically analyzed and printed out with the sample identification numbers.

The accuracy and reliability of the procedures, instrument and the reagents were ensured by quality controls. The quality controls have been performed before analyzing the samples, after replacement of reagents, at maintenance, if there is any doubt about the accuracy of the analyses of values and as required by the regulations. To ensure the accuracy of the test results, both the hematology and serum biochemistry analyzers were checked daily with quality control kits of known values for the different parameters.

RESULTS AND DISCUSSIONS

Clinical Parameters: The present study showed that the reference ranges of clinical parameters of donkeys are different from most of the previous reports [13,15, 16]. The differences could be due to breed, climatic factors, season, management, sample size and different methods used and this strongly signifies determination of these body parameters is critically important for each local species of animals in every country.

In horses and mules reports on their clinical parameters are very limited and the values are similar to the clinical values of donkeys [17]. Heart rate measurements during exercise have been used to describe the intensity of work, to measure fitness and to study the effects of training and detraining [18]. A direct relationship occurs between heart rate and amount of energy used during any given time. The 'true resting heart

rate', when the animal is resting quietly in a pasture will fall between 24 and 36 beats/min [19]. The differences in the ranges of these previous reports and the present study could be due to the differences in breed, management, age composition, exercise and training, season and other factors.

Hematological Parameters: The red blood cell count of donkeys was very similar to the previous reports [13, 15, 20-22]. However, the mean PCV of donkeys in this report (32.45%) was lower than those previously recorded [20] as 38% but similar to other previous reports.

Analyzing similar hematological parameters in a large population of donkeys (4000 donkeys), suggested that the differences obtained by other authors could be explained by inappropriate statistics on a small non-normal sample [13]. The hematological parameters of horses in this study were similar to those previously reported [23] and the serum biochemical reference ranges of horses were comparable with other reports [24]. Hematological values of horses were similar to the previous finding [23]. Female mules were not found in the study sites.

Serum Biochemical Parameters: Serum biochemical values of donkeys obtained in this study were different from those previously reported [13] but similar to those reported by other investigators [15, 20, 25]. These differences might be due to the differences in breed,

Table 1: Results of clinical parameters of study donkeys, horses and mules, Ethiopia

Species Parameters	Donkey (N=408)		Horse (N=50)		Mule (N=32)	
	Mean±sd	Range	Mean±sd	Range	Mean±sd	Range
Temperature [°C]	37.49±0.52	36-39.7	37.33±0.41	36.2-38.5	37.43±0.38	36.9-38.3
Resp. rate [br/minute]	40.89±9.07	16-66	38.8±7.89	24-60	36.88±7.09	28-60
Heart rate [b/minute]	51.65±6.93	36-80	48.04±6.67	26-64	45.88±7.87	32-60
Pulse rate [b/minute]	49.11±6.99	20-72	46.28±6.71	32-62	44±7.87	32-60
Capillary refill time[sec]	1.51±0.54	1-4	1.62±0.6	1-3	1.78±0.49	1-3
Gut sound[/minute]	1.81±0.77	1-4	1.72±0.7	1-3	1.91±0.78	1-4

Table 2: Results of hematological values of study donkeys, horses and mules, Ethiopia

Species Parameters	Donkey (N=315)		Horse (N=48)		Mule (N=31)	
	Mean±sd	Range	Mean±sd	Range	Mean±sd	Range
RBC [x10 ⁶ /μl]	5.47±0.73	2.89-7.92	6.91±1.18	4.22-11.59	6.74±0.92	5.2-8.86
PCV [%]	32.45±3.91	16.1-47.8	34.55±5.16	26.3-52.8	35.36±3.89	28.2-47.2
Hb[g/dl]	11.95±1.52	5.5-18.2	12.79±1.96	9.4-19	13.02±1.53	10.3-17.5
MCV [f/l]	59.6±3.82	49-70	50.33±4.06	41-64	52.64±3.98	44-60
MCH [pg]	21.93±1.52	18-25	18.63±1.64	14.9-23.2	19.44±1.65	15.9-23.1
MCHC [g/dl]	36.8±0.89	34-39	37.02±0.93	35-39	36.96±0.72	36-39
WBC [x10 ³ /μl]	14.54±3.47	7-25.6	11.19±2.74	7.2-18.8	10.73±2.26	7.3-16.3
Platelet [x10 ³ /μl]	281.15±108.73	18-681	207.65±51.7	42-325	254.77±61.35	123-378

Table 3: Results of serum biochemical values of study donkeys, Ethiopia

Species Parameters	Donkey (N=315)		Horse (N=48)		Mule (N=31)	
	Mean±sd	Range	Mean±sd	Range	Mean±sd	Range
AST/GOST [IU/l]	280.69±92.8	103-579	224.92±70.25	123-402	262.9±76.55	142-404
GPT/ALT [IU/l]	10.89±4.73	2-29	10.52±4.74	3-29	8.81±2.29	3-13
ALP [IU/l]	402.48±154.95	36-970	422.63±180.28	176-865	405.84±138.23	176-681
GGT [IU/l]	41.81±18.41	9-120	17.44±8.7	9-53	22.16±7.66	10-43
Creatinine [mg/dl]	1.1±0.2	0.25-1.74	1.23±0.23	0.81-1.73	1.35±0.33	0.95-2.45
Urea [mg/dl]	43.88±7.23	15-73	49.04±10.7	28-78	37.81±11.55	0-64
Glucose [mg/dl]	68.46±18.72	20-145	71.75±25.31	21-125	79.58±24.95	22-121
T. protein [g/dl]	7.97±1.29	2.61-13.91	7.5±1.09	5.37-10	7.83±0.79	6.3-10.4
T.bilirubin [mg/dl]	0.4±0.15	0-1.1	0.99±0.51	0.3-3.8	0.92±0.41	0.3-1.8
D.bilirubin [mg/dl]	0.21±0.11	0-0.7	0.62±0.3	0-1.7	0.57±0.33	0-1.6
Sodium [mmol/l]	132.92±5.64	115-167	134.35±6.19	124-150	134.16±6.66	117-149
Potassium[mmol/l]	4.51±0.48	2.8-6.2	4.24±0.68	2.9-6.3	3.93±0.63	3-6
Calcium [mmol/l]	0.79±0.22	0.05-1.39	0.88±0.2	0.3-1.3	0.7±0.23	0.05-1.1
Chlorine [mmol/l]	103.52±4.44	90-130	102.42±4.88	89-113	101.77±6.54	85-114
Triglycerides[mmol/l]	1.29±0.6	0.2-4.91	0.55±0.32	0.16-1.49	0.85±0.34	0.25-1.68

feeding management systems, seasons of study, sample storage mechanism, sample size, working conditions of the animals, techniques of analyses and reagent types. Comparison of the present results with other reports [20] does not show big variations; and any slight differences may be ascribed to differences in techniques. To compare the results of mule parameters there were no previously published works. The increase in urea level in adult donkeys might be due to the feed type and protein metabolism.

The variations in the serum biochemistry of working equids could be due to feeding differences, working conditions and other management factors as well as climatic factors. Serum calcium level in this study was lower than other reports [13]; this could be due to the fact that only ionized calcium level was considered in this study but the previous studies did not indicate whether it is total or ionized.

The serum enzyme levels of horses were slightly lower than those reported by others [26] and comparable to those in some studies [27]. The serum enzyme levels may vary due to the differences of the activities of the equines considered. In young horses K, Cl⁻ and total bilirubin were higher. Altitude had showed effects on AST, GGT, creatinine, urea, glucose, total protein, K, Ca⁺⁺ and total bilirubin levels in donkeys. Thus, differences in results among reports can probably be attributed to the differences in climatic conditions and to the irregular use of electrolyte supplementation during rides as well as feeding, training, exercise type and body condition [28].

CONCLUSIONS

Although the reference values of physiological parameters of equines and their quantitative and qualitative changes have been previously established, determination of these values for working equids in Ethiopia are virtually absent. In the present study, the clinical, haematological and serum biochemical values determined for working equids were different from previously known reference values of other parts of the world and this indicates that determination of these values of the indigenous animal species is important. The results obtained in the present study likely represented most working equids in Ethiopia and constitute potential reference values that can be used for clinical purposes against the already known values. The results were crucial in a day-to-day clinical activity, research works and academics in the area of working equids. Based on the conclusion the following general recommendation is forwarded:

- Because of the possible individual or combined influence of different factors other than age, sex and altitude and the subsequent interpretation of normal ranges in working equines for pathological condition should be carefully considered and determined separately.

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