

## Some Physiological, Hematological Values and ANAE-Positive Lymphocyte Rations of Domestic Donkeys (*Equus asinus*) in Kyrgyzstan

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### Abstract

The aim of this study was to determine the physiological, hematological parameters and ANAE positivity of donkeys in Kyrgyzstan. Animals (n=24) were clinically examined and blood samples were taken. The average pulsation and respiration rates of male and female donkeys were measured as 52.25±9.27; 49.16±4.80 beats/minute and 18.41±4.21; 18.58±3.30 breaths/minute, respectively. HGB, MCV, MCH and MCHC values did not differ in the groups. RBC and WBC values were higher in females. Mean ANAE-positive PBL ratio of donkeys was found as 42.90±1.18%. Consequently, some physiological, hematological values and ANAE-profile were determined and advised as reference values of donkeys in Kyrgyzstan.

**Keywords:** Physiology, Hematology, ANAE-positivity, Donkey, Kyrgyzstan

## Kırgızistan'da Barındırılan Evcil Eşeklerde (*Equus asinus*) Bazı Fizyolojik ve Hematolojik Değerler ile ANAE Pozitif Lenfosit Oranının Belirlenmesi

### Özet

Bu çalışmanın amacı, Kırgızistandaki eşeklerin fizyolojik, hematolojik değerleriyle ANAE-pozitiflik oranının belirlenmesidir. Hayvanların (n=24) genel klinik muayenesi yapıldı ve kan örnekleri alındı. Erkek ve dişi eşeklerin ortalama pulzasyon ve respirasyon oranları sırasıyla, 52.25±9.27; 49.16±4.80 atım/dk ve 18.41±4.21; 18.58±3.30 solunum/dk olarak ölçüldü. HGB, MCV, MCH ve MCHC değerlerinde gruplar arasında bir fark bulunamadı. RBC ve WBC değerleri dişilerde daha yüksek saptandı. Eşeklerin ANAE-pozitif PBL oranı %42.90±1.18 olarak belirlendi. Sonuç olarak, Kırgızistanda yaşayan eşeklere ait bazı fizyolojik, hematolojik ve ANAE-pozitiflik oranı belirlenmiş ve referans değer olarak sunulmuştur.

**Anahtar sözcükler:** Hematoloji, ANAE pozitifliği, Eşek, Kırgızistan

### INTRODUCTION

Determination of the normal physiological, hematological and biochemical parameters help evaluating the

clinical prognosis of many animal diseases <sup>[1,2]</sup>. In addition, alpha-naphthyl acetate esterase (ANAE) staining has been used as a useful tool to differentiate T and B lymphocytes and monocytes in some certain species including many



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animals and thought to be responsible for the cytotoxic effects of T lymphocytes<sup>[3,4]</sup>.

The aim of this study was to determine the normal physiological and hematological parameters and ANAE positivity profile of donkey which live and freely fed in Tong region of Kyrgyzstan, did not referred before, and present the reference values.

## MATERIAL and METHODS

### Animal Selection and Sample Collection Procedure

The animals in different ages and weights (143-170 kg) were selected living in Tong region, Kyrgyzstan (42°18'32.41» N; 76°17'10.33» E, 5350 ft). Donkeys (male, n=12 and female, n=12) were divided in to three groups according to their ages. They were 6-24 months aged (4 male, 7 female), 2-15 years aged (6 male, 3 female) and >15 years aged (2 male, 2 female). The study was done in November and average weather temperature was recorded between minus 6±2.5°C and plus 12±2.0°C during all days according to weather forecast<sup>[5]</sup>.

In order to detect healthy condition, general clinical examination methods were used and blood samples were taken.

### Physiological and Hematological Parameters

Donkey's rectal body temperatures were measured by digital thermometers (DIGI-TEMP, Kruuse). After the reaching comfort (15 to 30 min), respiration was noted and pulsation rates also measured by statoscopes.

Blood samples were collected by jugular vein via needle (1.2 mm X 38 mm) to heparinized tubes. Red blood cell (RBC) count, white blood cell count (WBC), hemoglobin

(HGB), hematocrit (HCT), blood clot cell count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were measured with Auto Hematology Analyzer (Mindray BC-5300, China).

### ANAE Demonstration and Evaluation

Air dried smears were fixed in phosphate buffered glutaraldehyde-acetone solution (pH 4.8) at -10°C for 3 min. ANAE demonstration was performed by according to Ozaydin et al.<sup>[6]</sup> The cells with lymphocyte morphology and having 1-3 large, reddish-brown granules were classified as ANAE-positive lymphocytes (*Fig. 1*) under the Nikon Eclipse 50i light microscope (Japan) by counting 200 lymphocytes.

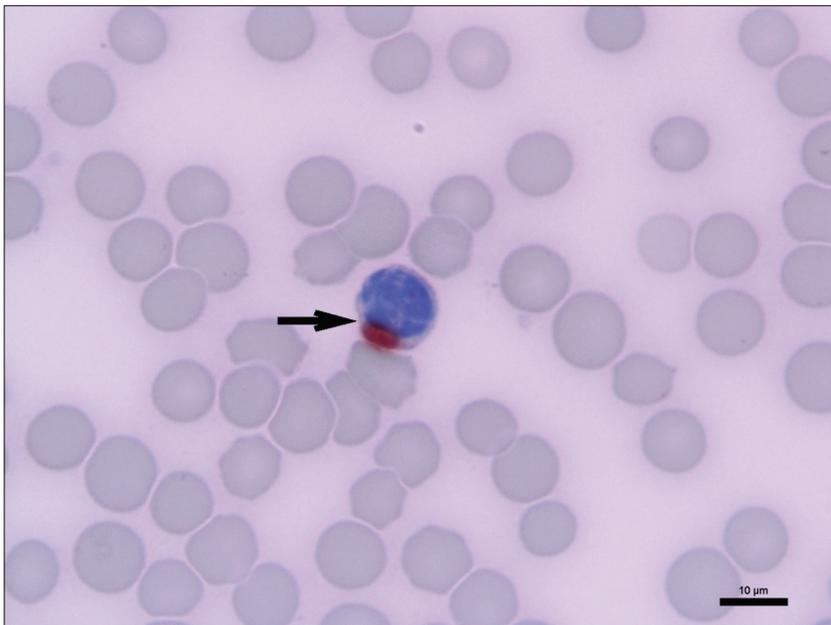
### Statistical Analysis

Data were analyzed using SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA) using sample t-tests to compare within groups and one way a nova 3 independent sample were used to compare between-group differences. A P-value <0.05 was considered statistically significant.

## RESULTS

### Physiological and Hematological Parameters

The average body temperature, pulsation values and respiration rates were determined as 37.60±0.62°C, 52.25±9.27 beats/minute, 18.41±4.21 breaths/minute for males and 37.03±1.34°C, 49.16±4.80 beats/minute and 18.58±3.30 breaths/minute for females, respectively. Similarly, RBC, WBC, HGB, HCT PLT, MCV, MCH and MCHC were analyzed for both sexes and statistical difference was not found within the groups (*Table 1, Table 2*).



**Fig 1.** ANAE-positive lymphocyte in the peripheral blood of donkey (*arrow*), ANAE demonstration, Barr: 10 μm

**Şekil 1.** Eşek periferik kanında ANAE pozitif lenfosit (*ok*), Bar: 10 μm

**Table 1.** The average blood parameters of male donkeys in different ages**Tablo 1.** Farklı yaştaki erkek eşeklerde ortalama kan parametreleri

Age	Male (n=12)							
	RBC (10 <sup>12</sup> /L)	WBC (10 <sup>9</sup> /L)	PLT (10 <sup>9</sup> /L)	HCT (%)	HGB (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
6-24 months aged	4.23	10.98	165.2	28.6	10.6	52.7	22.3	35.0
2-15 years aged	5.18	11.28	119.8	30.0	10.4	55.1	21.3	34.8
>15 years aged	5.42	10.37	158.5	29.8	11.1	57.8	20.0	35.7

**Table 2.** The average blood parameters of female donkeys in different ages**Tablo 2.** Farklı yaştaki erkek eşeklerde ortalama kan parametreleri

Age	Female (n=12)							
	RBC (10 <sup>12</sup> /L)	WBC (10 <sup>9</sup> /L)	PLT (10 <sup>9</sup> /L)	HCT (%)	HGB (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
6-24 months aged	5.24	11.8	137.1	28.4	9.98	54.8	19.0	35.1
2-15 years aged	5.04	11.6	122.6	30.3	10.4	60.1	19.7	34.5
>15 years aged	5.41	11.75	121.5	31.9	11.65	59.6	18.85	36.4

### ANAE Positivity

Mean ANAE-positive PBL (Fig. 1) ratio of donkeys was found as 42.90±1.18%.

## DISCUSSION

The average body temperature, pulsation values and respiration rates were determined as 37.60±0.62°C, 52.25±9.27 beats/minute, 18.41±4.21 breaths/minute for males and 37.03±1.34°C, 49.16±4.80 beats/minute and 18.58±3.30 breaths/minute for females, respectively. There was no statistical difference in the physiological parameters between and within the groups (P>0.05). Body temperature for many mammals under the normal conditions is regulated around 36-37°C [7,8]. The pulsation rate varies with age, size, and weight, activity or heavy physical work, excitement, anger and drug administration [9]. In our study, respiration rates of young male donkeys (6-24 months aged) was determined higher than the other groups but no statistical difference was found (P<0.05). Respiration rate can be changed according to the body weight, age, exercise, excitement environmental temperature, pregnancy, gastrointestinal fullness and diseases [10].

Many of our hematological data were supported by Laus et al. [11] WBC values were detected higher in female donkeys than males in our study similar with Babeker and Abdalbagi [12]. WBC values can be affected some intrinsic and extrinsic factors [13]. PLT and HGB values were determined higher in older groups (Above 15 years) in the present study. It was found that altitude is the most important factor affecting the reference value of the RBC and hematocrit [14].

MCV concentrations were observed higher in female groups than males. In contrary, MCH values were founded

higher in male groups than females in our study. Besides, MCV and MCH values were determined higher in older donkeys (above 15 years) in other studies [2,11]. MCHC values were determined same between male and female age groups in our study. Hence many factors such as environmental condition, diet, fasting, drugs administration might be affected on our different datas [12,15].

Although there is no knowledge of whether ANAE positivity is specific for T-lymphocytes of the donkeys in Kyrgyzstan our histochemical results may be beneficial for further immunological and functional studies.

Consequently, the average body temperature, the average pulsation values, the average respiration rates, some hematological values and ANAE-profile was determined and advised as reference values in domestic donkeys (*Equus asinus*) in Tong region of Kyrgyzstan.

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