Comparison of some hematological values and alpha-naphthyl acetate esterase (*ANAE*)-positive lymphocyte ratios of sheep breeds (Hissar and Edilbaev) reared in Kyrgyzstan

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Abstract: The aim of the study was to the compare the hematological parameters and ANAE-positive lymphocyte ratios of sheep breeds (*Hissar and Edilbaev*) which reared in Kyrgyzstan and present the reference values. Fifty (50) ewes (*Hissar*, n=20 and *Edilbaev*, n=30) were clinically examined and blood samples were taken. There were no difference on hematological parameters between the breeds (p>0,05). RDW-CV (red cell distribution width) and RDV-SD (standard deviation) values were found higher (p<0,05) in *Hissar* ewes. ANAE-profile were detected higher in *Edilbaev* ewes (p<0.01). Consequently, some hematological values and ANAE-profile were determined and advised as reference values.

Keywords: hematology, anae- positivity, sheep, kyrgyzstan

Kırgızistan'da Yetiştirilen Bazı Koyun Irklarının (Gisar ve Edilbayev) Hematolojik ve ANAE-Pozitif Lenfosit Oranları Yönünden Karşılaştırılması

Özet: Bu çalışmanın amacını Kırgızistan'da bulunan koyun ırklarının hematolojik ve ANAE-pozitif lenfosit oranları yönünden karşılaştırılması oluşturmuştur. Elli (50) dişi koyunun (*Hisar, n: 20 ve Edilbayev, n: 30*) ön klinik muayeneleri yapılmış ve kan örnekleri alınmıştır. Hematolojik parametreler yönünden gruplar arasında herhangi bir farklılık tespit edilememiştir (p>0,05). RDW-CV (red cell distribution width) ve RDV-SD (standard deviation) değerleri Hisar koyunlarında daha yüksek bulunmuştur (p<0,05). ANAE-profili ise Edilbayev koyunlarında yüksek bulunmuştur (p<0.01). Sonuç olarak, koyunlara ait kan parametreleri ve ANAE profile belirlenmiş ve referans değerler olarak sunulmuştur.

Anahtar sözcükler: hematoloji, anae- pozitiflik, koyun, Kırgızistan

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INTRODUCTION

Sheep breeding is the most important activity for economic, environmental, sociological and cultural status of the Kyrgyzstan. It provides raw material for industry which process animal products such as food, textile and hides (1). The most common sheep types are *Jaidara*, *Edilbaev* and *Hissar*. There is no current information about hematological parameters and ANAE-profile of sheep breeds except Jaidara (2) for this region.

Determination of the normal hematological parameters help evaluating the clinical diagnosis and prognosis of animal diseases (3). Besides, alpha-naphthyl acetate esterase (ANAE) staining has been used for differentiation of T, B lymphocytes and also monocytes in some animal species and reported to be responsible for the cytotoxic effects of T lymphocytes (4).

The aim of this study was to the compare the hematological parameters and ANAE positive lymphocyte rations of ewes (*Hissar* and *Edilbaev*) which reared in Kyrgzstan and present the reference values for this region.

MATERIAL and METHODS

Ethical approval and study location

This study was conducted with the approval of the Ethics Board of the Faculty of Veterinary Medicine of Kyrgyz-Turkish Manas University (No:20016/03-03). Fifty (50) clinically healthy (*Hissar*, *n=20* and *Edilbaev*, *n=30*), aged 2-5 years and of a mean body weight of 65-85 kg ewes which lived and freely fed in grasslands of three different locations of Kyrgyzstan were used. Besides, ewes were examined gynecologically and were not in estrus cycle or pregnancy. These study regions were Belovodsk (42° 49'49"N, 74°06'50"E, altitude 732 m) and Karabalta (42°49'22"N, 73°51'11"E, altitude 789 m). The study was performed on September 2016 and the average weather temperature was recorded between plus 33/11°C during all days according to weather forecast (5).

Sample collection

Blood samples were collected from the jugular vein with steril injector (0,8mm x 38mm) into anticoagulant-coated tubes (3ml) for the determination of some hematological parameters and ANAE-profile. The blood samples were transferred to the laboratory immediatelly after being collected and under cold chain conditions.
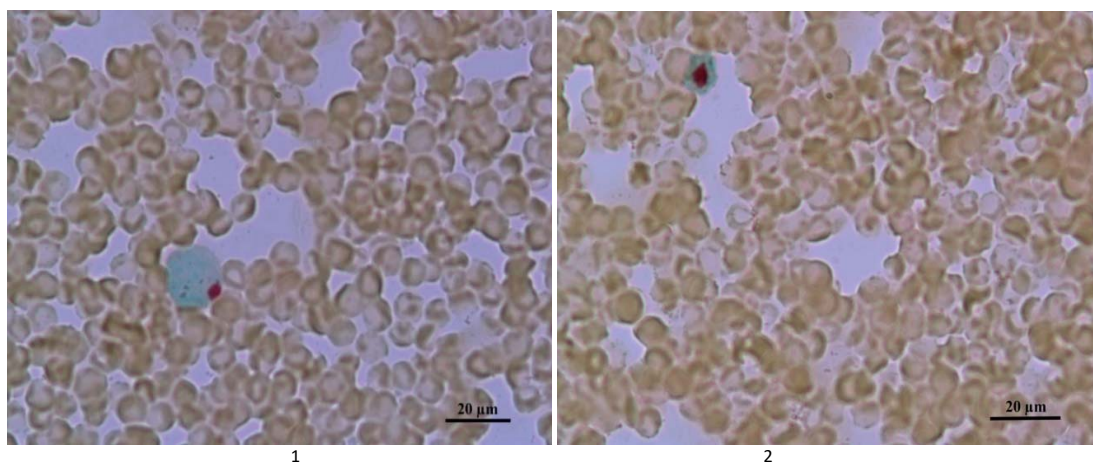
Hematological parameters

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).

Demonstration of alpha-naphthyl acetate esterase (ANAE)

Two smears were prepared from each blood sample. Smears (Air dried) were fixed in phosphate buffered glutaraldehyde-acetone solution (pH 4.8) at -10°C for 3 min. ANAE-profile demonstration was performed by according to Donmez *et a.l* (4). The cells which are with lymphocyte morphology and have 1-3 large, reddish-brown granules were determined as ANAE-positive lymphocytes (*Fig.1*) under the Nikon Eclipse 50i light microscope (Japan) by counting 200 lymphocytes.

Fig 1. ANAE-positive lymphocyte in the peripheral blood of *Edilbaev* (1) and *Hissar* (2) ANAE demonstration, Barr:20 μm

Statistical analysis

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

RESULTS

Hematological Parameters

WBC values were found higher in *Edilbaev* ewes. RBC, HCT, MCV, MCH, MCHC values were detected higher in *Hissar* ewes than *Edilbaev*. RBC, WBC, HGB, HCT, PLT, MCV, MCH and MCHC were analyzed and statistical difference was not found between the groups (*Table 1*). RDW-CV (red cell distribution width) and RDV-SD (standard deviation) values were determined higher in *Hissar* ewes than *Edilbaev* ($p<0,05$) (*Table 1*).

ANAE Positivity

ANAE-profile were detected higher in *Edilbaev* (52.65±1.78) than *Hissar* (46.42±2.45) ewes in statistic ($p<0.01$) (*Table 1*).

Table 1. Comparison of some hematological values and ANAE-positive lymphocyte ratios of sheep breeds with minimum and maximum levels.

Parameters	*HISSAR (n=20)*			*EDILBAEV (n=30)*		
	Mean	*Min/Max*		*Mean*	*Min/Max*	
WBC (109/L)	6.68±0.96	1.600	20.98	8.10±0.74	2.000	16.01
RBC (1012/L)	7.98±0.86	1.780	19.22	6.96±0.35	1.080	9.500
HGB (g/l)	10.80±1.00	2.200	26.00	10.50±0.56	2.300	17.00
HCT (%)	28.40±3.73	5.700	79.70	23.62±1.30	3.200	33.30
MCV (Fl)	34.57±0.51	32.00	41.50	33.66±0.22	30.50	36.00
MCH (pg)	14.81±0.68	12.30	25.30	13.61±0.60	2.300	16.80
MCHC (g/L)	43.70±2.24	37.50	78.80	42.91±1.68	10.20	70.00
RDW-CV (%)	15.23±0.32 a	13.30	19.10	14.52±0.15 b	13.30	17.10
RDV-SD (fL)	17.85±0.90 a	13.80	30.20	15.53±0.25 b	12.90	19.00
PLT (109/L)	496.8±63.36	91.00	1093	582.2±61.14	84.00	1300
MPV (f/L)	12.23±0.17	10.20	13.50	12.63±0.26	10.60	18.20
PDW	13.85±0.14	12.30	15.00	13.82±0.14	12.60	15.30
ANAE-P (%)	46.42±2.45 ab	20.00	62.00	52.65±1.78 a	36.00	70.00

a,b *p* < 0.05. Significant is important in the same sampling time between the groups.

Ihsan KISADERE, Nurcan DONMEZ, Nariste KADYRALIEVA, Abdulkadir KESKIN,

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DISCUSSION and CONCLUSION

WBC values were found higher in *Edilbaev* ewes as 8.10±0.74×103/mm3 in present study. WBC values of *Jaidara* ewes were determined for this region as 4.92±0.59x103/mm3 by Donmez et al. (2). These values were detected for *Tuj* and *Morkaraman* sheeps as 7.03-7.93 and 6.29-6.31 103/mm3 by another researchers, respectively (6). WBC values can be affected from infectious diseases, parasite infestations, failure of food or water intake, temparature extremes and also psychological disturbance (7, 8). RBC, HGB, HCT, MCV, MCH, MCHC values were observed lower in *Edilbaev* ewes. Although, HCT values (% 23.62±1.30-28.40±3.73) were found lower when compared also with *Merino* and *Awassi* sheeps, MCH and MCHC values were detected higher in our study groups than *Merino* and *Awassi* sheep breeds (9, 10). Hence many factors such as genetics, geographical location, season, day length, altitude, drugs administration might be affected on our different datas (11). Although, RDW-CV values were found higher in *Hissar* ewes as % 15.23±0.32 in present study, the high percent of RDW-CV values were determined as % 18.20±3.098 in *Tibetan* sheeps (12). It may be affected depend on seasonal variation, altitude or gender. However, PLT (platelet count) and platelet distribution width (PDW) values were observed higher in *Hissar* ewes, mean platelet volume (MPV) which is a measure of platelet size were detected higher in *Edilbaev* ewes. Our study group results were observed very high when compared with *Tibetan* and *Sangsari* sheeps by means of PLT and MPV values (12). As reported before, MPV cannot be considered separately from PLT count and these values might be affected from hormonal activity and gestational age (13). ANAE-positive lymphocyte ratio values were found statistically (p<0.01) higher in *Edilbaev* ewes. In our previous study percentages of ANAE-profile for Jaidara ewes was reported as % 65.00±2.55 that is very higher than *Hissar* and *Edilbaev* ewes (2). Besides, our present results for *Hissar* and *Edilbaev* were significantly lower from percentage of *Merino* (%73) and *Merino lamps* (% 67,7) (14). Sur (14) reported that the percentage of ANAE-positive lymphocytes can change in the periphery blood circulation depend on management and nutrition, factors effective on the health status of animals, and particularly by alterations in the immunity levels of animals. Although, there is no data of whether ANAE positivity is specific for T-lymphocytes of the sheep breeds in different locations of Kyrgyzstan, our histochemical results may be useful for further hematological, immunological and functional investigations.

Consequently, some hematological values and (ANAE)-positive lymphocyte ratios were determined and advised as reference values of ewes (*Hissar* and *Edilbaev*) reared in Kyrgyzstan.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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