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PAPESHE

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Genetic analyses of sheep populations

Project acronym: **PAPESHE**

Project full name: **Protection of Autochthonous populations of PElagonia SHEep breed in the cross-border area**

Start date of project: **30 July 2018**

Duration of project: **24 months**

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Key information

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Introduction

The reference level is considered as a value of a given parameter ranging the 95% of the animal population denoted as healthy animals. The reference range is of pivotal importance for screening and diagnostic tests aimed at evaluating health and disease in a given population. However, mild aberrations from the reference range registered in the major population could be characteristic for certain population and can be considered as a specific trait for that population and a degree of breed specificity. The attempt to be established a reference range for the indigenous sheep population on Balkan Peninsula, has been recorded by many author having in mind specifics for sheep population. Additionally, there are recommendations for testing reference ranges and internal valorization for each laboratory following the strict preanalytical, analytical and statistical laboratory rules.

Hematology evaluation could provide the useful and objective information, primary for the animal health, animal subjected to stress and also function of the certain organs and organic systems. Biochemical assessment abide the health status is also useful to determine the metabolic and nutritional status of the tested animal. The variations are mirrored by the many factors. Namely, statistically significant differences could be observed depending on living conditions, region, husbandry, rearing location, and especially diet. Differences concerning sex, reproductive status and age of the animal are considered as differences within the population.

Hematology and biochemical blood assessment was the aim of this investigation focused to determine possible specifics in content of the cellular and biochemical blood content giving that data on the hematological and biochemical parameters of indigenous sheep breed in Pelagonia region are scarce

Methodological approach

The analysis was conducted within period of two months. Sampled animals were fed pasture and meadow hay ad labium. Blood samples were taken from 5 (five) previously selected flocks/farms recognized as breeding autochthonous sheep breed. In total 50 samples were taken, 10 samples per flock. Sampled animals were randomly chosen and all of the animals were female gender. Blood samples were taken in volume of 10 ml per sample by puncture of the jugular vein. Samples for hematology analysis were collected in sterile vacuum tubes with anticoagulant (EDTA) to inhibit process of coagulation and samples for biochemical analysis, were collected in sterile vacuum tubes which were allowed to clot for at least 30 minutes prior centrifugation. Samples were centrifuged on 1500 rpm for 10 minutes after what serum was decanted in to sterile eppendorf tube and kept on -20^{0}C until analysis were performed.

Picture 1

Picture 2

Picture 3

Hematology and biochemistry analysis were performed on instruments specialized for these type of analysis. Instruments were installed in the laboratory established in the premises of Veterinary Faculty Bitola. Procured instruments are designed for veterinary use and

analysis can be performed on versatile animal species. Installation and training has been held prior starting the analysis proposed in this project (picture 4).

Picture 4

Blood cell count was evaluated with HumaCount 30ts suitable for veterinary use in versatile animal species (picture 5)

Picture 5

Biochemical analysis was performed with clinical chemistry autoanalyzer Chem 200 also for veterinary use, suitable for versatile animal species (picture 6). The instruments were founded within the frame of the project PAPESHE.

Picture 6

For hematology analysis following parameters were analyzed: white blood cell count, lymphocytes, concentration of haemoglobin, MCH and MCHC values, red blood cell count, MCV value, haematocrit and trombocytes count. Content of Ca, Mg and P, also concentration of albumin, cholesterol, creatinine, glucose, HDL, LDL, total protein, trygliceride and urea were determined in sera. Additionally the activities of the following enzymes were also assessed: GGT, ALP, AST, ALT, LDH.

Picture 7

Picture 8

Additionally specific laboratory equipment (consumables, disposable materials and reagents) has been procured also funded within the project. The disposals were mainly equipment for blood sampling and additional auxiliary materials. Reagents were also included for both instruments (picture 8). Biochemical analyzer was generally provided with the reagents required for obtaining data on metabolic profile in examined animals (picture 9).

Picture 9

Picture 9

Results and discussion

The results of hematology and biochemistry assessment are shown in Table 1, Table 2 and Table 3. According the blood cells count results, the values of analyzed parameters are within the reference range. Increased white blood cells were detected in some animals which is the indicative of presence of some infection. With the highest prevalence are the udder infection which is indicative of practicing lower level of hygiene during the milking and practicing the traditional methods of hand milking. However there are limited data on the incidence and prevalence of mastitis and several factors about the disease and leaves it susceptible to under-reporting. Foot diseases like footrot and lameness were also very often reported but the prevalence is not very high as it is with other sheep breed. The application of regular veterinary-medical measures, such as dehelmintisation or vaccinations, can be the reason for the appearance of health disturbances that are reflected on cell blood count and are most often the result of negligence of the breeder. However, according the data provided from the breeders and veterinarians in charged for investigated autochthonous flocks, the prevalence for common sheep pathology is lower compared to other sheep breed.

In this investigation content of the calcium in the animal serum, is above the reference range opposite the phosphorous which is lower compared to reference values according Kaneko at al (1997)[1]. The concentrations of the minerals are often influenced by the metabolic rate and homeostatic processes. But changes in concentrations are more related to animal nutrition and supplementation and also the concentration of minerals on the pasture.

Biochemical parameters are important in metabolic, nutritional and health status assessment of the animal. In this investigation increased albumin and urea concentration, slightly increased triglycerides with adequate concentration of total proteins, cholesterol, glucose and total bilirubin concentration were recorded compared to reference range. These changes may be linked with the biochemical structure of the pasture, especially the content of the proteins and also with the nutritional supplementation of the animals. Some authors have been reported that higher protein, urea and cholesterol concentrations in blood sera could be result of the water deprivation or water restriction [2]. Activity of liver enzymes and concentration of total billirubin accompanied with concentration of urea is indicative and recommended as reliable parameters for assessment of the liver status in animals.

Table 1: Hematology parameters

Indicators	unit	$\bar{x}$	σ	Reference values*
WBC	(10^9/L)	13.21	8.27	4.0-12.0
Lymphocytes	(10^9/L)	6.92	6.13	2.0-9.0
Haemoglobin	(g/dL)	9.98	1.45	9.0-15.0
MCH	(pg)	13.40	4.9	8.0-12.0
MCHC	(g/dL)	37.21	4.65	31.0-34.0
RBC	(10^{12}/L)	14.68	1.97	8.0-18.0
MCV	(fL)	41.98	4.12	28.0-40.0
Haematocrit	(%)	45.98	3.12	27.0-45.0
Trombocytes	(x10^9/ L)	446.07	26.45	250-750

*Jackson, P. G., (20020) [3]

Table 2: Biochemical and mineral concentration in the blood serum

Indicators	unit	$\bar{x}$	σ	Standard error	Reference values*
Albumin	g/dL	**3.04**	0.43	0.06	2.4-3
Bilirubin total	mg/dl	0.21	0.13	0.05	0.1- 0.499

Cholesterol	mmol/l	1.60	0.34	0.052	1.35-1.97
Creatinine	umol/l	74.12	11.00	1.71	50-109
Glucose	mmol/l	3.99	2.35	0.36	2.78-4.44
HDL-Cholesterol	mmol/l	1.08	0.16	0.03	-
LDL-Cholesterol	mmol/ll	0.58	0.09	0.014	-
Total proteins	g/dl	6.90	0.39	0.06	6-7.9
Triglyceride	mmol/l	**0.29**	0.06	0.01	0.0-0.2
Urea	mmol/l	**7.61**	1.87	0.30	2.86-7.14
Calcium	mmol\l	**3.16**	0.19	0.03	2.80-3.20
Magnesium	mmol/l	1.03	0.09	0.01	0.90-1.26
Phosphore	mmol/l	1.5	0.43	0.07	1.62-2.63

*Kaneko J., at al (1997) [1]

Table 3: Enzymes activity in blood serum

Indicators	**unit**	**x̄**	**σ**	**Standard error**	**Reference values***
ALP	U/l	260.39	15.47	25.28	68-387
GGT	U/L	51.06	11.98	2.05	20-52
AST	U/L	107.68	16.30	2.69	60-280
ALT	U/L	15.9	4.99	0.83	6-20
LDH	U/l	376.33	25.07	73.63	238-440

*Kaneko J., at al (1997) [1]

Conclusion

The results in hematology and biochemical assessment in indigenous breed in Pelagonia region are indicators on adequate supply of sheep with minerals, protein and energy. Some of the increased parameters are indicative on possible lack on water availability which is common when the nutrition is based on external concept of breeding by dominant usage of the natural food sources on remote distance on the field. Therefore, blood metabolic profiles are suggested to be used as a reliable criterion for the nutritional and health status assessment.

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[3] Jackson, P. G., Cockcroft, P. D., & Elmhurst, S. (2002). *Clinical examination of farm animals* (No. Sirsi) i9780632057061). Oxford: Blackwell Science.