Reference values for haematology and biochemistry parameters in the Pelagonian Pramenka sheep breed

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Abstract

The Ovcepolian type of Pramenka sheep is most prevalent in Pelagonia region. It is considered a valuable source of genetic diversity, characterised with a number of beneficial traits. Haematology and biochemistry measurements is important for health assessment of animals. The aim of this study was to determine the reference ranges for selected haematology and biochemistry parameters in the Pelagonian Pramenka sheep breed. The study was carried out on 55 clinically healthy, non-pregnant sheep, 1 to 3 years old, during spring season, from three different locations in the Pelagonia region, North Macedonia. Blood samples were collected from the jugular vein into vacuum tubes with an anticoagulant for haematological analysis and vacuum tubes free of anticoagulant for biochemical analysis. Reference values may be more adequate as it is later, for biochemistry parameters. Reference values for hematology parameters were: leukocytes, lymphocytes, mid-size cells, cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells, granulocytes, haemoglobin, haematocrit, erythrocytes, erythrocyte indices (average volume per litre, average haemoglobin in erythrocytes, average haemoglobin concentration per litre, and platelets). Reference values for biochemistry parameters were: total proteins, albumins, total bilirubin, total cholesterol, creatinine, glucose, HDL-cholesterol, LDL-cholesterol, triglycerides, urea, aspartate aminotransferase, alanine amino transferase, alkaline phosphatase, glutamyl transferase, lactate dehydrogenase, and the concentrations of calcium, magnesium and inorganic phosphate. Most obtained values were comparable to other strains of Pramenka sheep. There was a clear deviation of certain reference values compared to the literature. Further analysis should be conducted to determine the reference values in rams and lambs.

Key words: *sheep; reference value; haematology; biochemistry parameters*

Introduction

The Pramenka sheep breed has been recognised as the oldest and the most prevalent indigenous sheep breed in the Balkan Peninsula, with numerous known phenotypes. Three indigenous genotypes, declared and documented as indigenous

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breeds, Sharplaninian, Karakachanian and Ovcepolian sheep breeds, have been identified and registered in North Macedonia (Dzabirski et al., 2012). The Ovcepolian breed represents the population of the greatest significance, as it accounts for the largest share in the total population and is most frequently recognised as an indigenous breed of the Pelagonia region. Phenotype analysis and morphometric measurements of the autochthonous breeds reared in Pelagonia region, were consistent with those reported for Ovcepolian type of Pramenka (Dameski et al., 2021). The Pramenka breed is considered a valuable genetic source of diversity, characterised by various beneficial traits, and a number of measures have been taken towards the conservation and maintenance of its genetic potential (Ćinkulov et al., 2008). Good management practice requires health assessments, including the measurement of key haematological and biochemical parameters. Such analysis can provide useful information about animal health and nutritional status (Antunović et al., 2009). Variations of blood parameter values can be affected by metabolic adaptation, stress and health status of the animal (Pokhyl et al., 2024). Moreover, sex, breed and age can also have a significant influence on values. Some authors have emphasised the importance of determining reference limits for specific breeds due to questionable comparisons with reference values given in the literature (Vojta et al., 2011; Cincovic et al., 2020). Therefore, the aim of this study was to determine the reference intervals for selected haematological and biochemical parameters in this indigenous sheep breed reared in the Pelagonia region.

Material and methods

A total of 55, clinically healthy, non-pregnant sheep with an average age of 1 to 3 years were included in the study. All animals were randomly chosen from three herds reared in three villages located in the Pelagonia region: Germian (N21.5297°, E40.9196°); Dobroveni (N21.6032°, E40.9490°); and Skocivir (N21.6383°, E40.9731°). The diet for all animals was free grazing with the addition of hay and barely-based concentrate ration. Water was available *ad libitum*. The study was conducted during the spring season (May to June). Blood samples were collected aseptically from the jugular vein with approximately 10 mL into vacuum tubes with anticoagulant (Ethylenediaminetetraacetic acid, EDTA) for haematological analysis and vacuum tubes free of anticoagulant for biochemical analysis. Samples were stored at 4°C and transported to the laboratory. Haematological analyses were performed within 6 hours of sampling on a Humacount haematology analyser equipped with software for sheep blood measurement. The following parameters were measured: leukocytes (WBC), lymphocytes (LYM), mid-size cells (MID), cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells, granulocytes (GRA), haemoglobin (HGB), haematocrit (HTC), erythrocytes (RBC), erythrocyte indices: average erythrocyte volume per litre blood (MCV), average haemoglobin in erythrocytes (MCH), average haemoglobin per litre blood (MCHC), platelet count (PLT). Prior to performing the biochemistry analysis, blood samples were allowed to clot at room temperature within 3 hours of collection. Serum samples were obtained through centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Biochemical analysis was performed using a Chem 200 Gesan (Italy) analyser with compatible Gesan biochemical reagents. The following biochemical parameters were determined: total proteins (TP), albumins

(ALB), total bilirubin (TBIL), total cholesterol (TCHOL), creatinine (CRE), glucose GLU), HDL-cholesterol, LDL-cholesterol, triglycerides (TRI), urea (BUN), aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), glutamyl transferase (GGT), lactate dehydrogenase (LDH), and the concentrations of calcium (Ca), magnesium (Mg) and inorganic phosphate (P). Statistical analysis was performed using MedCalc Statistical Software version 17.5.5 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017) for descriptive statistics and estimation of reference intervals (RIs), coefficient of skewness and coefficient of kurtosis. Normality distribution of each parameter was estimated using the Shapiro-Wilk test. Suspected outliers were detected according the Tukey's method. Since the number of collected samples (x=55) was within the range $40 \leq x \geq 120$, a robust method was used for RI calculation with a 90% confidence interval (CI) (Friedrichs et al., 2012). For each RI, the bootstrap method was used.

For this study, ethics permit no. 03-9/2, 13 01 2023 was granted by the Ethics Committee of the Veterinary Faculty, University of St Kliment Ohridski Bitola, North Macedonia.

Results

The estimated reference values for the selected haematology and biochemistry parameters are summarised in Table 1 for the haematology profile and in Table 2 for the biochemical profile of the Pelagonian Pramenka sheep breed. Reference intervals (RIs) are presented with confidence intervals of 90% for all analysed parameters. Normal frequency distribution was estimated in MCV, HCT and PCT for haematology parameters and for most biochemical parameters (TP, ALB, TCHOL, CRE, BUN, TBil, ALP, gGT, AST, ALT, Ca and Mg). The most outliers were detected in HTC (6) and HDL (4) and P (4).

Discussion

The reference interval is of pivotal importance for clinical interpretation of laboratory test results. Pavlov et al. (2010) noted that a different approach to calculating RIs can produce different results, even up to 20%. Sample size is of great importance in calculating RIs. Since the sample size of this study was less than n=120, bootstrapping as an alternative method was used to determine 90% CI (Friedrichs et al., 2012). This statistical approach was in accordance to the methodology developed by Dimauro et al. (2009), which simplified the estimation of reference values from a small sample size. Proper statistical interpretation of the data is provided by determining the normality of the mean distribution. In this study, the Shapiro-Wilk test (Friedrichs et al., 2012) showed that not all parameters were normally distributed. The assumption of normality is not required for nonparametric and robust methods of analysis and this approach was also applied by Vojta et al. (2011) in determining reference values in smaller group of sheep.

The results of the analysed parameters were compared with the literature RIs for sheep including other Pramenka strains. Results for the WBC RI in Pelagonian Pramenka were consistent with RI for sheep (Baumgartner and Wittek, 2017). Higher RIs were reported for Lika Pramenka (5.6–17.0 x 10^9 L) by Shek Vugrovecki et al. (2017) and lower for Dalmatian Pramenka (3.02–15.64 x 10^9 L) by Šimpraga et al. (2013). A wider RI for LYM and narrower RI for HGB values were recorded in Pelagonian Pramenka compared to literature RIs for

Table 1. Descriptive statistics and 90% CI for the lower and upper reference values of selected haematology parameters in the Pelagonian Pramenka breed

Parameter units	RI Lower ref. value (90% CI)	RI Upper ref. value (90% CI)	Mean ± SD	Coeff. of Skewness	Coeff. of Kurtosis	Normality p	Outliers	Literature reference interval*
WBC 10^9/L	4.02 (2.73 – 5.32)	14.02 (13.06-14.98)	9.02**±3.8**	0.62	-0.61	No	0	4.0-12.0
LYM 10^9/L	1.47 (0..59-2.16)	8.32 (7.47 -9.02)	4.89**±1.2**	0.36	-0.96	No	0	3.5-6.9
MID 10^9/L	0.72 (0.46-1.31)	3.34 (2.82-3.82)	2.03**±0.9**	1.42	2.28	No	2	/
GRA 10^9/L	0.87 (0.20-1.57)	6.71 (5.81-7.50)	3.79**±1.7**	1.00	1.17	No	0	/
HGB g/dL	7.59 (7.07-8.15)	12.17 (11.53-12.86)	9.88**±1.3**	1.07	0.93	No	1	9.0-15.0
MCH pg	8.85 (7.54-10.17)	13.62 (12.30-14.96)	11.24**±1.4**	1.12	1.86	No	1	8.0-12.0
MCHC g/L	285.50 (277.21-293.35)	340.09 (330.57-348.39)	312.8**±15.2**	1.14	1.49	No	1	310.0-340.0
RBC 10^{12}/L	6.08 (5.34-6.90)	11.38 (10.58-12.13)	8.73**±1.56**	1.20	1.89	No	3	9.0-15.0
MCV fL	29.18 (28.33-30.04)	35.68 (34.83-36.54)	32.43**±1.97**	0.20	-0.66	Yes	0	28.0-40.0
HTC %	20.70 (18.66-22.75)	36.23 (34.19-38.27)	28.46±4.72	-0.78	2.46	Yes	6	27.0-45.0
PLT 10^9/L	145.83 (11.96-179.27)	457.15 (415.25-494.46)	301.49±90.37	0.42	-0.31	Yes	0	250.0-750.0

* Baumgartner and Wittek (2017)

Table 2. Mean and reference values for haematology parameters in different strains of the Pramenka breed from the literature

Parameter	Units	Dubrovnik Pramenka[1] Mean	Zeta Zuja Mean [1]	Lika Pramenka Mean [2]	Dalmatian Pramenka[3] Mean	Pirot Pramenka [4]
WBC	10^9/L	8.11	8.05	5.6-17.0	3.02-15.64	6.93
RBC	10^{12}/L	9.53	8.97	6.6-9.9	7.81-12.77	9.84
PLT	10^9/L	195.70	404.10	75-807	731	382.72
HGB	g/dL	10.74	10.03	7.45-10.47	7.81-13.47	10.31
MCH	Pg/L	11.32	11.19	9.9-11.9	9.54-11.00	11.68
MCHC	g/L	248.00	266.30	320-353	284-362	339.00
MCV	fL	48.72	42.15	28.80-35.80	28.60-34.80	34.4
HTC	%	0.43	0.38	0.22-0.31	0.24-0.41	30.37

[1] Antunovic et al. (2015), [2]Shek Vugrovecki et al. (2017), [3] Šimpraga et al. (2013), [4] Ružić-Muslić et al. (2021)

sheep (Baumgartner and Wittek, 2017). Regarding the different granulocyte fractions, analysis was performed on granulocytes and values were comparable with neutrophiles and other granulocyte fractions (eosinophils and basophils) and monocytes included in the parameter mid-range absolute count (MID). Similar results for HGB were reported for Lika Pramenka (7.45–10.47g/dL; Shek Vugrovecki et al., 2017) and Dalmatian Pramenka (7.81–13.47 g/dL; Šimpraga et al., 2013). Haematocrit RIs in Pelagonian Pramenka were lower than RIs for sheep, but similar to those for Lika (0.22–0.31%) and Dalmatian Pramenka (0.24–0.41). Up to a 46% HCT mean value was reported for the Tsigai breed (Antunović et al. 2009). The RI for RBC in Pelagonian Pramenka was lower than the literature RI for sheep (Baumgartner and Wittek, 2017). This is consistent with RIs for the Lika Pramenka and Dalmatian Pramenka strains. Regarding the clinical significance of RBC number in sheep, Žura Žaja et al. (2019) emphasised the importance of morphometric features of sheep erythrocytes due to their small size and possibility of analytical errors when analysed on haematology instruments calibrated for other species (Polizopoulou, 2010). Values for erythrocyte indices (MCH, MCHC and MCV) were comparable to RIs for sheep and those reported for other indigenous strains of Pramenka.

RIs obtained for most biochemical parameters for Pelagonian Pramenka were comparable to the literature RIs for sheep (Kaneko et al., 2008). The RIs for TP were 6.67–9.10g/dL in Lika Pramenka and 6.68–8.74 g/dL in Dalmatian Pramenka. For the Zeta Zuja breed, Antunović et al. (2011) reported a mean value up to 8.70g/dL of total protein. Stevanovic et al. (2015) reported 2.65–4.73g/dL for ALB in Karakacan Pramenka and 2.85–4.47 g/dL for Dalmatian Pramenka by Šimpraga et al. (2013). The RI for ALB in Pelagonian Pramenka was comparable to the RI for sheep (Kaneko et al., 2008), and the RI for GLU in sheep were also comparable to those for Pelagonian Pramenka. Values of 1.40–3.70 mmol/L GLU were reported for Lika Pramenka (Shek Vugrovecki et al., 2017) and 2.9–4.3 mmol/L for Dalmatian Pramenka (Šimpraga et al., 2013). A wider RI was determined for TCHOL in Pelagonian Pramenka, compared to the RI for sheep

Table 3. Descriptive statistics and 90% CI for lower and upper reference value of selected biochemical parameters in the Pelagonian Pramenka breed

Parameter units	RI Lower ref. value (90% CI)	RI Upper ref. value (90% CI)	Mean ± SD	Coeff. of Skewness p	Coeff. of Kurtosis p	Normality p	Outliers	Literature reference interval*
TP g/dL	6.25 (6.09-6.42)	7.54 (7.37-7.71)	6.89 ±0.39	0.29	0.37	Yes	1	6 – 7.9
ALB g/dL	2.64 (2.53-2.76)	3.54 (3.42-3.65)	3.09 ±0.27	-0.25	-0.51	Yes	0	2.4-3.0
GLU mmol/L	3.06 (2.91-3.20)	4.12 (3.97-4.27)	3.59 ±0.31	1.11	1.91	No	1	2.78 – 4.44
TCHOL mmol/L	1.10 (0.96-1.24)	2.16 (2.02-2.29)	1.63 ±0.32	0.01	-0.57	Yes	0	1.35-1.97
LDL mmol/L	0.42 (0.37-0.46)	0.75 (0.70-0.79)	0.58 ±0.10	0.09	-0.36	No	0	/
TRI mmol/L	0.08 (0.05-0.01)	0.35 (0.31-0.38)	0.21 ±0.01	0.97	1.01	No	1	0.0 – 0.2
HDL mmol/L	0.47 (0.31-0.65)	1.62 (1.44-1.79)	1.04 ±0.33	1.63	3.12	No	4	/
CRE µmol/L	57.14 (52.75-61.52)	90.85 (86.47-95.24)	73.99 ±10.24	-0.19	0.24	Yes	0	50 – 109
BUN mmol/L	4.21 (3.43-4.98)	10.16 (9.38-10.93)	7.18 ±1.79	-0.29	-0.27	Yes	0	2.86 – 7.14
TBil mg/dL	0.13 (0.10-0.16)	0.35 (0.32-0.37)	0.2 4±0.06	0.15	-0.63	Yes	0	0.1 – 0.5
ALP U/L	134.85 (103.24-166.47)	377.89 (346.28-409.51)	256.37 ±74.65	-0.08	-0.02	Yes	0	68 – 387
gGT U/L	30.44 (24.59-36.28)	75.38 (69.53-81.23)	52.91 ±13.06	0.08	-0.21	Yes	0	20 – 52
AST U/L	60.92 (55.96-66.55)	113.73 (107.53-119.37)	87.32 ±15.37	0.19	-0.78	Yes	0	60 – 280
ALT U/l	6.22 (3.80-8.63)	24.80 (22.38-27.21)	15.51 ±5.69	0.77	0.53	Yes	0	6 – 20
LDH U/L	163.50 (134.12-195.61)	470.12 (438.45-498.41)	316.81 ±19.78	-0.07	-1.27	No	0	238 - 440
Ca mmol/L	2.52 (2.44-2.61)	3.18 (3.09-3.27)	2.85 ±0.19	-0.83	0.62	Yes	1	2.80 – 3.20
P mmol/L	0.96 (0.82-1.09)	2.01 (1.87-2.15)	1.48 ±0.32	1.63	2.32	No	4	0.8 – 1.10
Mg mmol/L	0.87 (0.83-0.91)	1.18 (1.14-1.22)	1.02 ±0.09	-0.18	0.68	Yes	0	1.30 – 2.40

*Kaneko et al. (2008)

Table 4. Mean and reference values for biochemical parameters in different strains of the Pramenka breed from the literature

Parameter	Units	Dubrovnik Pramenka [1,2] Mean	Zeta Zuja [3] Mean	Karakacan [4,5] Mean	Lika Pramenka [6] Mean	Dalmatian Pramenka [7] Mean	Pirot Pramenka [8] Mean
ALB	g/dL	3.17[1]	3.06	2.65-4.73[4]	3.54-4.75	2.85-4.47	3.16
TBIL	µmol/L	2.60[2]	-	-	5.00-11.00	-	2.46
TCHOL	mmol/L	1.82[1]	1.84	-	0.74-2.47	-	1.99
CRE	µmol/L	90.08[2]	-	75[5]	74.5-103.2	98-144	59.18
GLU	mmol/L	3.63[1]	3.30	-	1.40-3.70	2.90-4.30	3.05
HDL	mmol/L	0.95[1]	0.93	-	-	-	-
LDL	mmol/L	0.73[1]	0.80	-	-	-	-
TP	g/dL	7.74[1]	8.70	4.90-7.60[4]	6.67-9.10	6.68-8.74	7.21
TRI	mmol/L	0.33[1]	0.26	-	-	-	-
BUN	mmol/L	5.08[2]	-	6.3[5]	6.70-10.9	3.50-7.80	4.57
ALP	U/L	363.20[2]	-	222.45[5]	-	-	-
GGT	U/L	49.50[1]	34.54	25.60-86.90[4]	-	-	41.19
AST	U/L	94.52[1]	118.17	65-172[4]	110.7-241	66.2-129.3	117.67
ALT	U/L	17.99[1]	18.80	6.5[5]	-	-	-
LDH	U/l	363.20[2]		495.25[5]	-	-	-
Ca	mmol/L	2.65[1]	2.68	2.15-3.22[4]	2.15-2.76	-	2.49
Mg	mmol/L	1.10[1]	0.96	-	-	-	0.86
P	mmol/L	1.68[1]	1.88	0.87-2.18[4]	1.32-2.39	-	1.28

[1,3] Antunovic et al. (2015), [2] Antunovic et al. (2011) [4] Stevanovic et al. (2015), [5] Bozhilova-Sakova and Dimitrova (2020), [6] Shek Vugrovecki et al. (2017), [7] Šimpraga et al. (2013), [8] Ružić-Muslić et al. (2021)

(Kaneko et al., 2008). Shek Vugrovecki et al. (2017) reported an RI of 0.74–2.47 mmol/L in Lika Pramenka. The HDL and LDL RIs in Pelagonian Pramenka were similar to mean values in Dubrovnik Pramenka (HDL 0.95 mmol/L and LDL 0.73mmol/L) and Zeta Zuja (HDL 0.93 mmol/L and LDL 0.80 mmol/L) (Antunović et al., 2015). Higher RIs for TRI, BUN and TBil were recorded compared to the literature RIs for sheep (Kaneko et al., 2008). The results for TRI were similar to mean TRI in Dubrovnik Pramenka (0.33 mmol/L; Antunović et al., 2015). Comparable values were recorded for AST, ALT and LDH in Pelagonian Pramenka with the literature RIs for sheep and mean values for other Pramenka strains. The RI values for gGT were higher in Pelagonian Pramenka compared to those for sheep in the literature (Kaneko et al., 2008) with a higher upper limit for gGT (25.60–86.90 U/L) reported for the Karakacan breed (Stevanovic et al. 2015). The RIs for Ca and P were comparable to the literature values for sheep (Kaneko et al., 2008) and with

other strains of the Pramenka breed. The Mg RI in Pelagonian Pramenka was lower than compared to the literature RIs for sheep, but comparable with mean values for Dubrovnik Pramenka, Zeta Zuja (Antunović et al., 2015) and Pirot Pramenka (Ružić-Muslić et al., 2021).

Conclusions

According the results obtained for the selected haematology and biochemistry parameters for the Pramenka breed reared in the Pelagonian region of North Macedonia, most values for reference intervals were found to be comparable with values reported for other strains of the Pramenka breed. Not all reference values can be compared with the reference values for sheep given in the relevant literature. Lower reference intervals were recorded for RBC and haematocrit but higher for TRI, BUN, TBil and gGT. A higher level of Mg was also recorded in Pelagonian Pramenka breed, though these values were comparable with other strains of the Pramenka breed. Thus, determining the RIs for the Pelagonian Pramenka breed is significant and useful in interpreting the health status of the Pelagonian Pramenka population. Further research on rams and lambs is required to determine the possible differences in reference intervals related to the age and sex of animals.

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Referentne vrijednosti za odabrane hematološke i biokemijske parametre u pelagonijske pramenka ovce

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Ovčepoljski tip pramenke najrašireniji u sjevernomakedonskoj regiji Pelagonija i smatra se vrijednim izvorom genetske raznolikosti obilježene različitim korisnim osobinama. Hematološko i biokemijsko mjerenje izuzetno je važno za procjenu zdravlja životinje. Cilj je ovoga članka bio utvrditi referentne raspone za odabrane hematološke i biokemijske parametre u pelagonijske pasmine ovce. Studija je provedena na 55 klinički zdravih, negravidnih ovaca u dobi od 1 do 3 godine starosti, tijekom proljeća, s tri različite lokacije u regiji Pelagonija. Prikupljeni su uzorci krvi punkcijom jugularne vene u vakuumskim epruvetama s antiokoagulansom (etilendiamintetraoctena kiselina EDTA) za hematološku analizu i vakuumskim epruvetama, bez antikoagulansa, za biokemijsku analizu. Izmjereni su referentni parametri za: hematološke parametre (leukociti - bijele krvne stanice), limfociti (LIM), stanice srednje veličine (MID), stanice koje odgovaraju monocitima, eozinofilima, bazofilima, blastima i drugim prekursorskim bijelim krvnim stanicama; granulociti (GRA), hemoglobin (GHB), vrijednost hematokrita (HTC), eritrociti (crvene krvne stanice), indeksi eritrocita; prosječan obujam eritrocita u litri krvi (MCV), prosječna količina hemoglobina u eritrocitima (MCH), prosječna koncentracija hemoglobina u litri krvi (MCHC), broj trombocita (PLT)); biokemijske parametre: ukupne bjelančevine (TP), albumini (ALB), ukupni bilirubin (TBIL), ukupni kolesterol (TCHOL), kreatinin (CRE), glukoza (GUK), HDL-kolesterol, LDL-kolesterol, trigliceridi (TRI), urea (BUN), aspartat-aminotransferaza (AST), alanin-aminotransferaaza (ALT), alkalna fosfataza (ALP), glutamil transferaza (GGT) i laktat dehidrogenaza (LDH) i udio kalcija, magnezija i anorganskih fosfata. Većina statistički dobivenih vrijednosti za referentne intervale usporediva je s vrijednostima dobivenima za druge sojeve pramenke. Uz to, postoji očit otklon određenih referentnih vrijednosti u usporedbi s referentnim vrijednostima za ovce koje se spominju u relevantnoj svjetskoj literaturi. Potrebno je provesti dodatnu analizu za utvrđivanje referentnih vrijednosti u ovnova i janjadi.

Ključne riječi: *ovca, referentna vrijednost, hematologija, biokemijski parametri*