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Breed, age, and sex-related variations in hematological and some biochemical parameters in the Tornjak dog

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Abstract: There is an increasing significance for the breed, age, and sex-related variations in hematological and biochemical parameters in veterinary medicine. The primary objective of this study was to identify possible breed-related differences and to determine the effect of age and sex on hematologic and serum biochemical parameters in the Bosnian and Herzegovinian – Croatian Shepherd dog Tornjak raised in Bosnia and Herzegovina (B&H). Data from 19 hematological and 17 serum biochemical parameters were analyzed in the group of 100 dogs (47 females and 53 males), sexually intact and aged between one month and twelve years. Breed affected TP (total protein) and ALB (albumin) value. The median TP value was slightly above the reference interval and the ALB median value was in the upper limit. Variations in hematological and serum biochemical parameters were 6 sex-related and 23 age-related. This study adds valuable insight into the specifics of the interpretation of laboratory findings in a small population of the indigenous Tornjak dog breed.

Key words: Tornjak, Bosnia and Herzegovina, blood, biochemistry, hematology, value

1. Introduction

Nowadays, each dog breed represents individuals with high levels of phenotypic homogeneities, reduced genetic diversity within breeds, and greater genetic divergence between breeds [1]. Strong selection of dogs imposed by breeders to create a homogeneous population of individuals with common morphological and behavioral traits probably led to many changes in the genome, including breed-specific variations in hematological and biochemical blood parameters [2].

The genetic mapping of loci affecting canine blood phenotypes by a genome-wide association study (GWAS) identified genome significant breed-specific associations for hematological and biochemical blood parameters. The strongest breed-specific genetic association of biochemical parameters was observed for alanine aminotransferase (ALT), fructosamine, and glucose, and of hematological parameters, segmented neutrophils, monocytes (MONO), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) [3,4]. Analysis of 6046 dogs revealed breed-specific differences in hematological measurements, suggesting a genetic contribution to inter-breed variations for these phenotypes [2].

Hypercholesterolaemia and glycemia in Shetland sheepdogs [5], thrombocytopenia in Cavalier King Charles Spaniels [6], and serum enzyme activity, cholesterol level, some hematological parameters in Bernese Mountain dogs [7] have been reported as breed-related variations.

The breed appears to be an important factor for the relevance of population-specific reference intervals and should be considered when performing health examinations involving hematologic and biochemical parameters. Other factors are influencing the hematological and biochemical parameters such as environment, the season of the year, and lifestyle are known as external factors and internal factors such as age, sex, and neutering status [8, 9, 10].

These factors illustrate the importance of conducting breed-specific research to identify the genetic and environmental contribution to hematological and biochemical parameters, which would contribute to the improvement of veterinary practice.

In B&H, there is one autochthonous shepherd dog breed, the Bosnian and Herzegovinian-Croatian shepherd dog (Tornjak), which evolved with the development of agriculture.

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The Tornjak ancestors were brought with nomadic cattle breeders from the east, of which are descendants of the ancestors of the Molossus breed [11]. According to International Canine Federation (FCI) breeds nomenclature and standards, the Tornjak is classified in Group 2 as a Molossian Mountain dog type[1]. In addition to conservation strategies, it is necessary to know the physiological characteristics of autochthonous breeds in the conditions in which they live and thus contribute to their conservation.

There have been no attempts to determine the values of biochemical and hematological parameters in the Tornjak dog breed. The objectives of this study were to examine hematological and some biochemical parameters in healthy Tornjak dogs raised in B&H, compare them to the standard reference intervals for all dogs, and assess the influence of age, breed, and sex on these parameters.

2. Materials and methods

Healthy Tornjak dogs with known pedigree status and owner consent were recruited for this study. During sampling, dog owners provided pedigrees with data on five generations of ancestors. Pedigree records are issued by the Union of Cynological Associations of Bosnia and Herzegovina**.** The Tornjak dog is usually bred for herding livestock, but the dogs included in this study were mostly companion animals. It is a large, strong, and agile dog breed, that can weigh up to 65 kilograms and be tall about 70 centimeters. The dogs live outside in agricultural areas with a continental climate and an altitude of 400–1000 m. Each dog was determined to be clinically healthy based on standard clinical procedures (anamnesis and physical examination). Dogs were excluded from the study if they were pregnant, had any known disease, or were receiving medication at the time of the examination (other than antiparasitic treatments in the past 3 months) that could interfere with blood analysis.

Blood samples were collected from June to November 2020 from 100 dogs of both sexes (47 females and 53 males), sexually intact and aged between one month and twelve years, in B&H. All dogs were grouped into puppy (≤ 1), young adult (1–3,9 years), adult (4–7,9 years), and senior (8–12 years) age groups.

Blood samples were collected in the morning before feeding and watering. The dogs fasted 12 h before sampling. The cephalic venous blood was obtained using a Vacutainer system into plain (4 mL) and K3-EDTA (2mL) sample tubes (VACUETTE, Greiner Bio-one, Hannover, Germany). Blood samples were collected as a part of the routine screening process and all animals were treated in accordance with Internal Ethical Committee (Number: 01-02-739-2/21) by respecting animal welfare and well-being. The K3-EDTA sample was used directly, while the serum tube was centrifuged at 1600 g for 15 min, separating the serum for further analysis.

Hematological analysis was performed using a stabilized K3-EDTA whole-blood sample on an IDEXX ProCyte Dx Hematology Analyzer (ProCyte Dx Hematology Analyzer, Idexx Laboratories, Westbrook, ME). The following parameters were measured: red blood cell parameters (red blood cell count - RBC, haematocrit - HCT, haemoglobin - HGB, mean cell volume - MCV, MCH, MCHC, red blood cell distribution width - RDW, reticulocytes - RETIC, reticulocyte haemoglobin - RETIC-HGB); white blood cell parameters (white blood cell -WBC count, neutrophils - NEU, lymphocytes - LYM, MONO, eosinophils - EOS, basophils –BASO); platelet parameters (platelet count – PLT, platelet distribution width - PDW, mean platelet volume - MPV, plateletcrit – PCT).

Serum was used for biochemical analysis on an in-house dry chemistry analyzer (Catalyst One, Idexx Laboratories Inc., USA). The following parameters were measured: glucose - GLU, creatinine - CREA, urea – UREA, blood urea nitrogen/creatinine ratio - BUN/CREA, phosphorus – PHOS, calcium - CA, TP, ALB, globulin - GLOB, albumin/globulin ratio - ALB/GLOB, alanine aminotransferase - ALT, alkaline phosphatase - ALKP, gamma-glutamyltransferase - GGT, total bilirubin - TBIL, cholesterol - CHOL, amylase - AMYL, lipase - LIPA.

2.1. Statistical analysis

Statistical analysis was done using IBM SPSS, version 21.0 (IBM Corp., 2012). Data were evaluated by the Shapiro–Wilk test to determine distribution. Initial analysis showed that the value distributions in most of the examined parameters were significantly different from normal, with the presence of one or more atypical (extreme) values. Therefore, nonparametric statistical tests were applied in further processing. The Mann–Whitney U test was used to detect the significance of differences between two groups, and the Kruskal–Wallis test was used to compare three or more groups. Obtained p values less than 0.05 were considered statistically significant. After the Kruskal–Wallis test, post hoc analysis was performed using the Mann–Whitney U test with prior adjustment of the significance level using Bonferroni correction according to the pattern p = alpha level/number of groups for comparison, p = 0.05 / 4 in the case of comparison of age categories, and the obtained result of p = 0.0125 was used as the level of significance.

3. Results

This study focused on a hematological and serum biochemical evaluation of clinically healthy Tornjak dogs. Descriptive statistics of hematological and serum

[1] FCI (2017): http://www.fci.be/en/nomenclature/BOSNIAN-AND-HERZEGOVINIAN-CROATIAN-SHEPHERD-DOG-355.html (Accessed 18.05.2021.)

biochemical parameters with reference intervals for dogs [12, 13] can be found in Table 1 and Table 2. A total of 19 hematological and 17 biochemical parameters were determined.

Median values of all analyzed hematological parameters were within the reference interval considered physiological for dogs [13].

Median values of most analyzed biochemical parameters were within the reference interval considered physiological for the dogs [12]. The median value of the TP was slightly above the reference interval and the ALB median value was in the upper limit.

3.1. Age-related changes in hematology and serum biochemistry

Hematology: A significant effect of age (Table 3) was present for RBC, HCT, HGB, and all RBC indices (MCV, MCH, MCHC), leukocytes (LYM, MONO, EOS), and platelet indices (MPV, PDW, PCT).

Serum biochemistry: A significant age effect (Table 3) was present for most of the parameters except for UREA, BUN/CREA, ALT, TBIL, and CHOL.

3.2. Sex-related changes in hematology and serum biochemistry

Hematology: Sex-related changes in hematological parameters (Table 4) were identified in PLT and PCT values.

Serum biochemistry: Sex-related changes in serum biochemical parameters (Table 4) were identified in ALT, TBIL, CHOL, and LIPA values.

4. Discussion

The Tornjak dog is recently recognized by the FCI. Researches on the Tornjak dog breed are limited. For now, exterior body characteristics, microsatellite diversity of the breed, and the incidence of hip dysplasia are investigated [14[2], 15, 16[3]].

The current study demonstrates hematological and serum biochemical parameters with the influence of breed, age, and sex in the clinically healthy Tornjak dog breed population. The literature contains hematological and serum biochemical reference intervals of canine species and individual breeds. Still, this subject has not been investigated in the Tornjak dog breed population.

In our study, there was no evidence of a difference in hematological parameters between the Tornjak dog breed and the reference interval for the normal dog [13].

Tornjak dogs showed a higher median TP, slightly above reference interval for dogs, and ALB median value was in the upper limit. Median values of other determined biochemical parameters in this study were within the reference interval considered physiological for the dogs [12].

The influence of breed on TP value was established in some studies [17] but not in others [7, 18].

Higher concentrations of TP in intact dogs in both sexes compared to neutered ones were noticed, which probably represents the influence of sex hormones and reduced clearance of these parameters in the neutered state [8]. This could explain the value of TP in the Tornjak dog sample because all individuals in this study were intact.

Values of ALB reported by other authors [8, 17, 18] were similar to the result obtained in this study.

Serum ALB concentration may be an indicator of body protein status [19]. High upper limits of TP and ALB in the population of adult Bernese Mountain dogs were also reported [7]. Tornjak is a large breed dog, which could explain the elevated values of ALB and TP.

The influence of age on RBC, HGB, HCT median values, and RBC indices MCHC and MCH in this study was present. Parameters were lowest during the puppy life stage.

Median values of RBC, HGB, and HCT obtained in this study were in agreement with results reported by Harper, Rosset, and Rørtveit [20, 21, 22]. Lower values of these parameters are normal for puppies since erythropoietin production decreases in the early postnatal period, but they will reach adult values until 12 months of age [20, 23]. A similar pattern of values of these parameters is normal due to their physiological relationship [2].

MCHC values did not change with age in some studies [2, 20, 21], but in others, the results were similar to ours [22].

Lawrence reported lower MCH in dogs aged ≤ 1 and then an increase after puppy age, which was consistent with our result [2]. Lower MCH values in puppies were published by Rosset [21]. Puppies have a lower HBG concentration, as well as MCH value due to the maturation of erythropoiesis and a transition from fetal to postnatal erythrocytes [2].

The influence of age on RBC, HGB, HCT median values, and RBC indices MCHC and MCH were absent in other studies [5, 9, 24].

In this study, leukocyte values were influenced by age. The median LYM values were highest in puppies and young adults, MONO in puppies, and EOS in young adults and adults. Despite the higher count of some WBCs in younger Tornjak dogs in the present study, this does not appear to be attributable to bacterial infection, as dogs have been found to be healthy during clinical examinations. These results

[2] https://www.cabdirect.org/cabdirect/abstract/20143253096

[3] https://www.cabdirect.org/cabdirect/abstract/20123313733

Table 1. Results of descriptive statistics for hematological parameters of the Tornjak dog breed compared with the Reference interval for dogs.

Variable	N	Mean	StDev	Min	Max	Median	IQR	Q1	Q3	RI for dogs*
RBC (x10^{12}/L)	100	7.35	1.20	4.48	9.58	7.52	1.73	6.46	8.19	5.50 – 8.50
HCT (%)	100	47.03	7.96	28.60	62.00	47.25	11.75	41.03	52.78	37.00 – 55.00
HGB (g/dL)	100	16.39	2.90	8.90	21.40	16.50	4.05	14.43	18.48	12.00 – 18.00
MCV (fL)	100	64.03	2.88	53.60	70.30	64.45	3.38	62.63	66.00	60.00 – 77.00
MCH (pg)	100	22.28	1.28	17.10	24.10	22.40	1.60	21.70	23.30	**21.20-25.90
MCHC (g/dL)	100	34.80	1.21	30.30	38.10	34.90	1.00	34.50	35.50	32.00– 36.00
RDW (%)	100	18.45	2.30	14.20	28.50	18.40	2.40	17.10	19.50	**13.60-21.70
RETIC (%)	100	1.00	1.18	0.20	6.90	0.60	0.80	0.40	1.20	0.00 – 1.50
RETIC-HGB (pg)	100	21.93	1.66	15.10	25.70	22.15	1.90	21.0	22.90	
WBC (x10^9/L)	100	13.00	2.89	7.57	20.97	12.63	3.78	10.90	14.68	6.00 –17.00
NEU (x10^9/L)	100	7.50	2.34	3.57	14.48	6.80	2.95	5.68	8.63	3.00 –11.50
LYM (x10^9/L)	100	3.25	1.13	1.15	5.91	3.12	1.69	2.38	4.08	1.00 – 4.80
MONO (x10^9/L)	100	0.86	0.35	0.39	2.10	0.76	0.38	0.62	1.00	0.15 – 1.35
EOS (x10^9/L)	100	1.36	1.05	0.03	7.13	1.02	0.79	0.78	1.57	0.10 –1.25
BASO (x10^9/L)	95	0.04	0.04	0.01	0.20	0.02	0.03	0.01	0.04	Rare
PLT (K/µL)	100	251.70	95.03	55.00	462.00	255.00	125.25	188.50	313.75	200.00 –500.00
MPV (fL)	100	11.41	1.65	8.40	17.80	11.00	1.98	10.30	12.28	8.70 – 13.20
PDW (fL)	88	12.13	1.88	8.60	16.80	12.05	2.85	10.70	13.55	9.10 – 19.40
PCT (%)	100	0.28	0.10	0.06	0.57	0.27	0.13	0.21	0.34	0.14 – 0.46

N - number of valid observations; StDev – standard deviation; Min – minimum; Max – maximum; IQR – interquartile range; Q1 – lower quartile; Q3 – upper quartile; RI for dogs – reference interval for dogs.
*Rizzi et al. [13]; **Reference Intervals for the IDEXX ProCyte Dx* Hematology Analyzer

were in agreement with results reported by Bourgès-Abella comparing 9 and 36 months old laboratory beagles [25[4]]. Harper published a significantly higher WBC count in puppies [20].

The decrease in WBC count with age, particularly LYM, is a reflection of immunosenescence, a decline in immune function with aging [10, 26, 27]. The decline in LYM is typically credited to a shift of T-cells to memory cells and changes in the mitotic capacity of cells [27]. The EOS count could be higher due to parasites, but also in allergic and anaphylactic reactions [2].

The age did not affect LYM, MONO, and EOS values in other studies [2, 5, 9, 22].

In our study, MPV, PDW, and PCT median values were lowest in young adults and highest in puppies and senior dogs. On the contrary, these parameters were not affected by age in other studies [5, 9, 28] or were significantly higher during the first months of life [29].

An increased MPV value is associated with thrombopoietin concentrations, the primary regulator of megakaryopoiesis. Also, puppies are likely to become aroused during blood sampling and stimulate the release of platelets from the spleen which will affect all platelet indices [29].

The effect of age was recorded on 12 serum biochemical parameters in this study.

The median value of GLU was significantly higher in Tornjak puppies. These results were in agreement with results reported by Rørtveit [22]. No age-related difference was found in other studies [8, 21]. Previous studies reported the age effect on GLU values, but they did not include dogs younger than one year [9, 10, 27, 28]. The period of fasting, dietary changes, possible parasitic infestations, delayed separation of the serum from the blood clot might lead to a decrease in blood glucose concentration [21, 22]. Furthermore, glycogen reserves decrease with age in dogs, and after feeding old dogs it takes longer for blood glucose levels to reach baseline levels than in young dogs [30]. In fasting neonates, the liver controls and maintains blood glucose through glycogenolysis and gluconeogenesis.

[4] https://www.ingentaconnect.com/content/aalas/jaalas/2015/00000054/00000001/art00003

Table 2. Results of descriptive statistics for some serum biochemical parameters of the Tornjak dog breed compared with the Reference interval for dogs.

Variable	N	Mean	StDev	Min	Max	Median	IQR	Q1	Q3	Reference interval for dogs*
GLU (mmol/L)	100	4.94	0.83	3.13	6.69	4.98	1.17	4.38	5.55	3.61–6.55
CREA (µmol/L)	100	75.82	41.05	11.00	407.00	73.00	34.75	56.00	90.75	44.20–132.60
UREA (mmol/L)	100	6.94	3.89	1.50	21.50	6.35	4.40	4.08	8.48	0.60 – 1.60 **2.50–9.60
BUN/CREA	100	28.85	24.06	5.00	122.00	20.00	21.75	14.00	35.75	
P (mmol/L)	98	1.70	0.58	1.02	3.54	1.49	0.60	1.29	1.89	0.84–2.00
Ca (mmol/L)	100	2.59	0.17	2.18	3.16	2.59	0.25	2.47	2.72	2.25–2.83
TP (g/L)	100	73.61	11.90	51.00	120.00	72.00	12.75	67.00	79.75	54.00- 71.00
ALB (g/L)	100	33.25	5.85	23.00	60.00	32.00	7.00	30.00	37.00	26.00–33.00
GLOB (g/L)	98	39.87	6.72	26.00	60.00	40.00	7.50	35.75	43.25	27.00–44.00
ALB/GLOB	98	0.84	0.15	0.50	1.30	0.90	0.23	0.70	0.93	
ALT (U/L)	100	62.29	50.98	10.00	309.00	50.50	49.00	31.00	80.00	21.00 -102.00
ALKP (U/L)	99	72.96	88.70	10.00	459.00	40.00	60.00	25.00	85.00	**23.00 -212.00
GGT (U/L)	100	3.72	6.09	0.00	35.00	1.00	5.75	0.00	5.75	1.20–6.40
TBIL (µmol/L)	100	11.60	22.62	2.00	145.00	5.00	5.00	4.00	9.00	1.71–8.55
CHOL (mmol/L)	97	5.75	1.64	2.39	13.37	5.38	1.61	4.77	6.38	3.50–6.99
AMYL (U/L)	97	744.90	215.20	246.0	1309.00	724.00	311.50	583.50	895.00	185.00–700.00 **500.00 -1500.00
LIPA (U/L)	98	919.40	506.30	14.90	3759.00	771.00	563.00	600.80	1163.80	13.00 –200.00 **200.00 – 1800.00

N- number of valid observations; StDev – standard deviation; Min – minimum; Max – maximum; IQR – interquartile range; Q1 – lower quartile; Q3 – upper quartile; RI for dogs – reference interval for dogs; *[12]; **Reference Intervals for the IDEXX Catalyst One* Chemistry Analyzer.

These processes are unsuppressed under hyperinsulinemia in newborns [31].

Median values of CREA obtained in our study were lowest in puppies. Our results were in agreement with results reported in previous studies [8, 21, 22]. A correlation between CREA concentration and age was not established in some studies [5, 9, 27]. The reason for the lower CREA value in puppies could be due to less muscle mass compared to body size in young animals [22]. In our study, a significant decrease in median CREA value was present in seniors, which is consistent with results obtained in other studies [8, 28]. The decrease of CREA value in the senior life stage can be attributed to age-related sarcopenia [8].

Median values of P and Ca in our study were highest in puppies and lowest in seniors. Age-related decreases in P and total Ca concentration have been previously published [8, 20, 22, 28]. The results of several studies in the adult dog population during their lifetime showed no age-related changes [5, 9, 27]. Variation in mineral concentration may have arisen due to increased bone metabolism in growing dogs, particularly large breeds [22]. Later skeletal maturation results in a decrease in serum minerals [10]. There are other proposed reasons for changes in Ca concentration, impaired intestinal Ca absorption during aging due to vitamin D deficiency [32]. It was suggested that a diet rich in protein, calcium, and phosphorus influences growth [20].

Values of TP, ALB, and GLOB in the present study were significantly lower in Tornjak puppies. Lower values of TP [8, 20, 21], ALB, and GLOB [8, 20, 21, 22] in puppies have previously been published. The lower TP, ALB, and GLOB values may occur due to incomplete liver maturation in puppies [21]. TP and GLOB values increased with age, and ALB values significantly decreased in seniors in our study. The continuous increase in TP and GLOB values may reflect changes in the globulin fraction of proteins, a phenomenon of 'inflammaging' due to chronic inflammation with age caused by a lifetime antigenic challenge resulting in the production of inflammatory proteins [26]. The ALB value was negatively associated with age in our study and this result was observed in other studies [8, 10, 24, 28].

Table 3. Hematological and some biochemical parameters influenced by age in the Tornjak dog breed.

	Puppy (n = 17)	Young adult (n = 41)	Adult (n = 30)	Senior (n = 12)	p value
RBC (x10^{12}/L)	5.75 (1.32)^{b}	7.59 (1.32)^{a}	7.66 (1.46)^{a}	7.51 (1.37)^{a}	< **0.001**
HCT (%)	38.10 (11.05)^{b}	49.70 (9.15)^{a}	49.55 (8.92)^{a}	46.30 (8.08)^{ab}	< **0.001**
HGB (g/dL)	12.90 (4.95)^{b}	17.50 (3.00)^{a}	17.15 (3.50)^{a}	16.00 (2.58)^{ab}	< **0.001**
MCV (fL)	63.90 (4.50)	65.30 (2.60)	64.00 (2.48)	62.70 (3.60)	**0.042**
MCH (pg)	21.60 (2.75)^{b}	22.70 (1.35)^{a}	22.55 (1.33)^{ab}	22.35 (1.70)^{ab}	**0.012**
MCHC (g/dL)	33.90 (3.20)^{b}	35.10 (0.95)^{a}	35.30 (1.33)^{a}	34.80 (0.60)^{a}	< **0.001**
LYM (x109/L)	3.99 (1.64)^{a}	3.14 (1.85)^{a}	2.58 (1.04)^{bc}	2.09 (2.15)^{c}	< **0.001**
MONO (x109/L)	1.10 (0.56)^{a}	0.71 (0.41)^{b}	0.65 (0.20)^{b}	0.96 (0.49)^{ab}	< **0.001**
EOS (x109/L)	0.71 (1.07)^{ab}	1.21 (0.85)^{a}	1.07 (0.94)^{a}	0.75 (0.32)^{b}	**0.002**
MPV (fL)	11.80 (2.50)^{a}	10.40 (1.65)^{b}	11.50 (2.55)^{a}	11.90 (1.53)^{a}	< **0.001**
PDW (fL)	11.90 (3.40)^{ab}	11.00 (2.90)^{b}	12.60 (3.20)^{a}	13.80 (1.60)^{a}	**0.001**
PCT (%)	0.34 (0.18)^{a}	0.26 (0.12)^{b}	0.23 (0.16)^{b}	0.32 (0.13)^{ab}	**0.017**
GLU (mmol/L)	5.87 (1.19)^{a}	4.95 (1.01)^{b}	4.84 (1.35)^{b}	4.47 (1.78)^{b}	< **0.001**
CREA (µmol/L)	60.0 (31.0)^{b}	78.0 (39.50)^{a}	77.50 (22.25)^{a}	64.00 (38.50)^{ab}	**0.031**
P (mmol/L)	2.56 (0.87)^{a}	1.58 (0.41)^{b}	1.32 (0.65)^{bc}	1.29 (0.33)^{c}	< **0.001**
Ca (mmol/L)	2.74 (0.14)^{a}	2.59 (0.21)^{b}	2.57 (0.25)^{b}	2.42 (0.18)^{c}	< **0.001**
TP (g/L)	61.00 (11.50)^{b}	72.00 (8.50)^{a}	77.00 (12.50)^{a}	72.50 (11.25)^{a}	**0.001**
ALB (g/L)	30.00 (5.50)^{b}	34.00 (7.00)^{a}	32.00 (7.25)^{ab}	29.00 (4.50)^{ab}	**0.010**
GLOB (g/L)	31.00 (3.00)^{b}	40.00 (5.50)^{a}	42.00 (8.25)^{a}	43.50 (9.25)^{a}	< **0.001**
ALB/GLOB	0.90 (0.20)^{a}	0.90 (0.25)^{a}	0.80 (0.20)^{ab}	0.70 (0.18)^{b}	< **0.001**
GGT (U/L)	5.00 (9.00)^{a}	0.00 (3.50)^{b}	1.00 (6.25)^{ab}	0.00 (3.00)^{ab}	**0.033**
ALKP (U/L)	123.00 (148.80)^{a}	36.00 (45.50)^{b}	35.50 (25.25)^{b}	36.50 (307.00)^{ab}	**0.001**
AMYL (U/L)	520.00 (259.00)^{b}	717.50 (255.30)^{a}	740.00 (324.30)^{ab}	841.50 (305.80)^{a}	**0.003**
LIPA (U/L)	633.00 (212.00)^{b}	956.00 (598.00)^{a}	781.50 (735.80)^{a}	693.00 (505.00)^{ab}	**0.015**

Data are presented as median (IQR). Medians in the same row with different superscript are significantly different; IQR – interquartile range.

Increased production of globulin proteins over a lifetime can lead to decreased ALB production [24], as well as to a loss of muscle cells [30].

In our study, the enzyme activity was affected by age. The ALKP and GGT activity was the highest, and AMYL was the lowest in puppies. The LIPA activity was highest in young adults and adults.

The high ALKP activity was recorded in a group of Borzoi, Beagle, Labrador Retrievers, and mixed breed puppies [20, 21, 22]. Increased osteoblastic activity during bone growth is the result of high serum ALKP activity [33]. Age-related changes in ALKP and GGT activity were not recorded in studies involving dogs older than one year [5, 9, 10, 28].

No age-related differences in results of serum AMYL and LIPA activity were found in several reports [22, 28]. Renal insufficiency may result in increased serum AMYL and LIPA activity due to catabolism in the kidney [34], but no dog has shown a clinical sign suggestive of renal problems.

Sex-related differences in hematological parameters detected in the study were recorded in PLT and PCT values and were higher in females.

Increased PLT counts have been previously noted in female dogs [2, 10, 29]. Lack of sex effect was present in Shetland Sheepdogs, Dogues de Bordeaux, and Greyhounds [5, 28, 35]. A pathway of estradiol synthesized in megakaryocytes has been identified, that triggers a proplatelet formation, but this is sex-independent autocrine steroid action for platelet production [36]. The role of estradiol in megakaryocytopoiesis was confirmed in vitro by administering 17beta estradiol to CD34(+) cells [37]. However, in the human population, women had higher PLT than men, but this data did not change after

Table 4. Hematological and some biochemical parameters influenced by sex in the Tornjak dog breed

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	M (n = 47)	F (n = 53)	**p value**
PLT (K/μL)	235.00 (129.00)	272.00 (122.50)	**0.010**
PCT (%)	0.24 (0.14)	0.30 (0.12)	**0.016**
ALT (U/L)	36.00 (39.00)	59.00 (41.00)	< **0.001**
TBIL (μmol/L)	5.00 (2.00)	6.00 (9.00)	**0.012**
CHOL (mmol/L)	5.03 (1.14)	5.91 (1.51)	**0.014**
LIPA (U/L)	649.50 (381.80)	970.00 (726.50)	**0.006**

Data are presented as median (IQR).
IQR – interquartile range; M-male; F-female.

menopause. This implies that estrogen has a small role in megakaryocytopoiesis. Authors suggest an estradiol role in conjunction with genetic polymorphisms [38].

The value of platelet indices PCT in other studies did not show a difference between males and females [5, 28, 29]. Higher PCT in females in this study is probably related to the breed.

Sex-related differences in serum biochemical parameters obtained in the study were recorded in ALT, TBIL, CHOL, and LIPA values, and were higher in females.

Higher serum ALT activity in males was reported in other studies [10, 39, 40], or no sex-related difference was detected [5, 28]. A significant age-sex correlation in ALT activity has been determined [10]. The serum ALT activity was increasing in females with age. In our study, the median values were within the reference interval of the general canine population in both groups and no clinical signs associated with liver damage were detected, the finding may be irrelevant.

Our result of TBIL value was in agreement with results published by Connolly [10], but sex difference was not recorded in a study conducted by Chang [8].

Higher CHOL and LIPA values in females were also detected in other studies [39, 40, 41]. This was explained by lower hepatic LIPA activity in females, which is inconsistent with the result obtained in our study, LIPA activity was higher in Tornjak females [41].

Chang et al. [8] imply a possible influence of male sex hormones on LIPA activity. In their study, LIPA activity was notably lower in intact than in neutered male dogs, but there were no differences in intact and neutered female dogs. Intact male dogs showed the lowest LIPA activity [8].

In conclusion, the results obtained in this study showed that age and sex had a stronger effect than breed on the investigated parameters in clinically healthy Tornjak dogs. There are 6 sex-affected and 23 age-affected hematological and serum biochemical parameters. This study adds valuable insight into the specifics of the interpretation of laboratory findings in a small population of the indigenous Tornjak dog breed. This research is the first hematological and serum biochemical study on Tornjak dogs that could help veterinarians avoid misinterpreting laboratory results in the diagnostic process, prognosis, and therapeutic monitoring.

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