

## THE INFLUENCE OF AGE AND GENDER ON HEMATOLOGICAL AND SOME BIOCHEMICAL PARAMETERS IN BOSNIAN MOUNTAIN HORSE

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**Abstract.** The aim of the present study was to investigate values of hematological and some biochemical parameters of autochthonous Bosnian mountain horse breed by revealing the differences related to age and gender. These horses are adapted to specific environmental and climatic conditions in the region and their blood parameters might differ from other horse breeds.

Blood samples were collected on stud farm "Borike" from 30 clinically healthy adult horses of both gender (16 females and 14 males), aged between two and twenty-three years. Horses were grouped in three age classes: young mature horses (2-6 years, n=10), middle age horses (7-14 years, n=10) and old horses (>14 years, n=10). A total of eleven hematological and twelve biochemical parameters were analyzed.

Gender significantly affected LYM (p=0.009), EOS (p=0.016), UREA (p=0.018), P (p=0.028), TBIL (p=0.048) and AMYL (p=0.033) levels. Values of UREA, TBIL and AMYL were significantly higher in males, while values of LYM, EOS and P were significantly higher in females. Age significantly affected GLU (p=0.019) and UREA (p=0.001) levels. Values of GLU were significantly lower in middle age horses than in old horses while values of UREA were significantly lower in young mature horses than in middle age and old horses. UREA was the only parameter affected by both age and gender. Results obtained in the present study showed that gender had much more powerful effect on the investigated parameters in clinically healthy adult Bosnian mountain horses than did the age.

**Keywords:** Bosnian mountain horse, blood, biochemistry, hematology, value

**Introduction.** Bosnian mountain horse (Bosnian and Herzegovinian mountain horse, Bosnian pony), the only autochthonous horse breed in Bosnia and Herzegovina is an internationally recognized ancient breed that belongs to "warm-blooded" horses (Dekic et al., 2014). The breed has long been created by crossing between the tarpan (*Equus caballus gmelini*) and the Asian wild horse (*Equus caballus przewalskii*). Further infusions of oriental stock have probably been introduced to the breed by the Turks during the Ottoman Empire (Žiga and Telalbasic, 2009). Bosnian mountain horse has been prized in its habitat for many centuries and it has been selectively bred since 1900s. The principal breeding center has been the Stud farm "Borike" at the altitude of 950 m. These horses are adapted to specific environment and climatic conditions in the region and have characteristics that might be reflected in their hematological and serum biochemical parameters. Bosnian mountain horse is frequently used for light farm work, light draft, pack and riding and is very surefooted on a terrain unsuitable for motor vehicles (Dekic et al., 2014).

Hematological and serum biochemical parameters in horses are used for the clinical diagnosis of systemic, infectious and some parasitic diseases, recovery and therapeutic monitoring. It is important in postoperative

patients and it is used to assess the metabolic condition of a single animal (Mikniene et al., 2014).

Horses of different age, sex, breed and blood type may have a different range of blood hematological and biochemical parameters. Also, the blood profile of horses can be influenced by their temperament, which classifies horses as "hot-blooded" (HB), "warm-blooded" (WB) and "cold-blooded" (CB) (Mikniene et al., 2014). The hematological and biochemical values obtained abroad may not be fully applicable under local conditions because these are influenced by multiple factors, such as breed, environmental and management differences. Also, some variations may exist in results between laboratories using different reagents, methods and instruments (Gul et al., 2007).

Hematological and serum biochemical parameters in horses were investigated and published by many authors (Čebulj-Kadunc et al., 2002; Gupta et al., 2002; Čebulj-Kadunc et al., 2003; Lacerda et al., 2006; Gul et al., 2007; Altinsaat, 2008; Pritchard et al., 2009; Jagrić et al., 2012; Mikniene et al., 2014; Pađen et al., 2014; Freeman and Klenner, 2015). The effects of age and gender on some basic hematological parameters in Bosnian mountain horses are available in literature (Dekic et al., 2014).

However, no data of the effect of age and gender on serum biochemical parameters have been published.

**The objective of this study was** to investigate values of hematological and some biochemical parameters in clinically healthy adult Bosnian mountain horses by revealing the differences related to age and gender and to compare obtained results with results previously reported for other breeds of HB, WB and CB horses, and with reference intervals for adult horses in general.

#### Materials and methods

Blood samples were collected on stud farm Borike in May 2014, from 30 clinically healthy adult horses of both gender (16 females and 14 males) with ages between two and twenty-three years. Mares were kept outdoor on pastures of a mountainous plateau. During the winter they are also mostly outdoors and fed with hay. Stallions were kept in stalls. All horses were grouped in three age classes: young mature horses (2-6 years, n=10), middle age horses (7-14 years, n=10) and old horses (>14 years, n=10). Applying molecular markers were found that sampled horses are constituted of autochthonous Bosnian mountain horses and no genetic differentiation between them was observed (Rukavina et al., 2015).

Based on anamnesis and physical examination horses were clinically healthy. Trias (temperature, pulse and respiratory frequency), adspaction, palpation, auscultation and percussion were made.

Blood samples were collected in the morning before feeding and watering. The samples were obtained by jugular vein puncture (*vena jugularis externa*) in Vacutainer tubes with anticoagulant (EDTA) for hematological studies and in Vacutainer tubes with gel for biochemical analysis. In the blood samples collected with EDTA the number of red blood cells (RBC) ( $\times 10^{12}/L$ ), hematocrit (Hct) (%), hemoglobin concentration (Hb) (g/dl), mean corpuscular hemoglobin concentration (MCHC) (g/dl), platelet cells (PLT) ( $\times 10^9/L$ ), total number of leukocytes (WBC) ( $\times 10^9/L$ ), neutrophils (NEU) ( $\times 10^9/L$ ), lymphocyte (LYM) ( $\times 10^9/L$ ), monocyte (MON) ( $\times 10^9/L$ ), eosinophils (EOS) ( $\times 10^9/L$ ) and basophils (BASO) ( $\times 10^9/L$ ) were determined using an automated cell counter (IDEXX Laboratories, Netherland).

Blood serum was separated by centrifugation at 1600 g for 20 min. The serum samples were stored at  $-20^{\circ}C$  until further analysis. Concentration of glucose (GLU) (mmol/L), urea (UREA) (mmol/L), creatinine (CREA) ( $\mu\text{mol}/L$ ), phosphor (PHOS) (mm/L), calcium (Ca) (mmol/L), total protein (TP) (g/L), albumin (ALB) (g/L), globulin (GLOB) (g/L), alkaline phosphatase (ALP) (U/L), total bilirubin (TBIL) ( $\mu\text{mol}/L$ ), cholesterol (CHOL) (mmol/L) and amylase (AMYL) (U/L) were processed using standard procedures with The Catalyst One<sup>TM</sup> Chemistry Analyzer (IDEXX Laboratories, Netherland), using tests for IDEXX.

Descriptive statistics were computed for each of the blood parameters analyzed. Further statistical analysis to assess the effects of age and gender was performed using GLM procedure of Minitab 17 statistical software (Minitab, 2014), with post hoc Tukey test for age classes or t-test for gender comparison. P values less than 0.05 were considered statistically significant.

#### Results

Results of the analyzed hematological parameters in clinically healthy adult Bosnian mountain horses of different ages and gender are shown in table 1. A total of eleven hematological parameters were determined. Gender significantly affected LYM (p=0.009) and EOS (p=0.016) counts that were significantly higher in females than in males. Differences between age classes for all investigated hematological values were not significant.

Results of serum biochemical parameters are presented in table 2. A total of twelve biochemical parameters were determined. Age significantly affected GLU (p=0.019) and UREA (p=0.001) levels. Values of GLU were significantly lower in middle age horses than in old horses while values of UREA were significantly lower in young mature horses than in middle age and old horses. Gender significantly affected UREA (p=0.018), P (p=0.028), TBIL (p=0.048) and AMYL (p=0.033) levels. Values of UREA, TBIL and AMYL were significantly higher in males, while values of P were significantly higher in females. The only parameter affected by both age and gender was UREA.

Table 1. Hematological parameters in clinically healthy adult Bosnian mountain horses of different ages and gender

Parameter	Age (years)				Gender		
	2-6	7-14	>14	p=	Female	Male	p=
RBC ( $\times 10^{12}/L$ )	9.27	8.35	7.40	ns	8.83	8.15	ns
Hct (%)	44.22	42.98	34.90	ns	42.63	42.09	ns
Hb (g/dl)	15.83	15.17	12.80	ns	15.42	14.53	ns
MCHC (g/dl)	35.76	34.06	36.67	ns	35.39	34.56	ns
WBC ( $\times 10^9/L$ )	9.14	10.06	7.57	ns	9.84	8.18	ns
PLT ( $\times 10^9/L$ )	190.50	206.00	306.70	ns	223.50	187.00	ns
NEU ( $\times 10^9/L$ )	5.66	5.83	4.34	ns	5.53	5.63	ns
LYM ( $\times 10^9/L$ )	2.21	2.80	1.85	ns	2.77	1.61	0.009
MONO ( $\times 10^9/L$ )	0.64	0.62	0.59	ns	0.68	0.50	ns
EOS ( $\times 10^9/L$ )	0.61	0.77	0.75	ns	0.83	0.40	0.016
BASO ( $\times 10^9/L$ )	0.02	0.04	0.05	ns	0.03	0.04	ns

Table 2. Serum biochemical parameters in clinically healthy adult Bosnian mountain horses of different ages and gender

Parameter	Age (years)				Gender		
	2-6	7-14	>14	p=	Female	Male	p=
GLU (mmol/L)	5.15 <sup>ab</sup>	4.28 <sup>b</sup>	6.62 <sup>a</sup>	0.019	5.11	4.63	ns
UREA (mmol/L)	6.14 <sup>b</sup>	8.90 <sup>a</sup>	9.33 <sup>a</sup>	0.001	7.34	8.89	0.018
CREA ( $\mu$ mol/L)	124.60	97.80	122.00	ns	108.00	120.70	ns
P (mm/L)	0.92	0.92	0.86	ns	0.98	0.77	0.028
Ca (mmol/L)	2.98	2.89	2.89	ns	2.95	2.89	ns
TP (g/L)	70.22	75.10	70.33	ns	71.67	74.14	ns
ALB (g/L)	27.89	29.40	30.33	ns	29.00	28.71	ns
GLOB (g/L)	42.33	45.60	40.00	ns	42.67	45.29	ns
ALP (U/L)	174.10	160.80	194.67	ns	181.00	149.10	ns
TBIL ( $\mu$ mol/L)	18.44	24.80	23.33	ns	18.93	28.57	0.048
CHOL (mmol/L)	2.67	2.39	2.62	ns	2.52	2.57	ns
AMYL (U/L)	34.44	33.60	30.33	ns	29.40	42.29	0.033

<sup>a,b</sup> – Means in the same row (age) with different superscript are significantly different ( $p < 0.05$ ); ns - nonsignificant

### Discussion and conclusions

The present study investigated the influence of age and gender on hematological and some biochemical parameters in clinically healthy adult Bosnian mountain horses. The values of basic hematological parameters (WBC, RBC, Hct, Hb, MCV, MCH, and MCHC) of Bosnian Pony from the Borike Stud are available in literature (Dekic et al., 2014). However, effects of age and gender on serum biochemical parameters in Bosnian mountain horses have not been investigated before. Dekic et al. (2014) reported that stallions had significantly higher values of all RBC, Hct and Hb concentration than mares. In contrary, we found that differences between gender classes for investigated hematological values were not significant. Obtained differences could be due to sample size, different used methods, reagents and instruments.

Mean values of RBC, Hct and Hb, obtained in this study, were higher (insignificant) in mares than in stallions. These results were in agreement with results reported by Mikniene et al. (2014) for Žemaitukai horses. On the other hand, Čebulj-Kadunc et al. (2002) found the RBC, Hct and Hb mean values were significantly higher in stallions than in mares of WB horses. Both Čebulj-Kadunc et al. (2002) and Mikniene et al. (2014) reported lower WBC mean values in mares than in stallions, however, our results indicated the contrary. Our results of higher MCHC and WBC values in mares than in stallions were detected in previous study on horses (Jain, 1986). Mean values of LYM and EOS counts, obtained in our study, were significantly higher in mares than in stallions ( $p=0.009$ ;  $p=0.016$ , respectively). Higher values for EOS count in mares were also detected in Posavina and Croatian Coldblood horses (Pađen et al., 2014). According to Pađen et al. (2014) this result can be explained in the way that mares kept in an open area are continuously exposed to various parasite infestations. On the contrary, stallions are kept in stalls.

The mean values of RBC, Hct, Hb and WBC in our study were higher in young mature and middle age horses than in old horses. Our results were in agreement with results reported in previous studies (Gul et al., 2007; Mikniene et al., 2014). We considered higher MCHC values in old horses. Significantly higher MCHC values in older Lipizzan horses were published by Čebulj-Kadunc et al. (2002). According to Mikniene et al. (2014) lower RBC values in old horses could have been compensated by means of an increased erythrocyte size, which caused higher MCHC values. Satue et al. (2009) found higher PLT values in younger horses. Contrary, in this research PLT values exceeded the reference range for adult WB horses after 14 years of age. According to Satue et al. (2009) the results of previous research on PLT values are controversial and some authors found a reduction in PLT values in old horses while other researchers did not find any significant change connected with aging.

Values of GLU in the present study were significantly higher in old horses than in middle age horses ( $p=0.019$ ). GLU values exceeded the reference ranges for adult horses in general and adult WB horses after 14 years of age. Contrary to our results of highest GLU values in old horses, Zinkl et al. (1990) reported significantly lower GLU levels in older donkeys. Generally, older animals have lower GLU values and our results were in disagreement. This variation may have arisen from breed, environmental conditions, nutrition, as well as timing of blood sampling.

In the present study UREA was affected by both age and gender. Mean values of UREA were significantly higher in middle age and old horses than in young mature horses ( $p=0.001$ ). Our results were in agreement with results published by Čebulj-Kadunc et al. (2003) who found higher values of UREA concentration in older categories of Lipizzan horses. In this study stallions had significantly higher mean values of UREA than mares ( $p=0.018$ ). Contrary to our findings, Pađen et al. (2014)

reported higher levels of UREA in mares. Values of UREA exceeded the reference range for adult horses in general and adult WB horses in middle age and old horses.

In Bosnian mountain horses CREA concentration was numerically higher in stallions than in mares (120.70 vs 108.00  $\mu\text{mol/L}$ , respectively). Higher values of CREA concentration in stallions were published for Lipizzan horses (Čebulj-Kadunc et al., 2003). Contrary, Pađen et al. (2014) found significantly higher values of CREA in mares. Gupta et al. (2002) and Pritchard et al. (2009) found that CREA values increased with age, while Čebulj-Kadunc et al. (2003) detected the highest CREA concentration in foals. In this work, age related difference was not significant.

Significantly higher values of P concentration in mares ( $p=0.028$ ) were detected in this work. No age related difference was found, but we noticed that P concentrations decreased with age. This result was in agreement with Mikniene et al. (2014) who also found that P concentrations decreased with age in Žemaitukai horses. Age-related decrease in P is probably a reflection of decreased bone metabolism as animals grow older.

Čebulj-Kadunc et al. (2003) determined the highest Ca concentrations in stallions. Also, Pađen et al. (2014) detected significantly lower values of Ca in mares. Contrary to that, we did not find gender related differences in Ca concentrations. Ca concentrations were observed to be similar in all age groups as reported earlier (Gupta et al., 2002).

Gupta et al. (2002) and Pađen et al. (2014) found significantly higher ALB concentrations in stallions. In our investigations, no gender or age related differences were found.

We detected numerically higher ALP activity in mares. The same was obtained for Criollo horses (Lacerda et al, 2006) and Posavina and Croatian Coldblood horses (Pađen et al., 2014). Pađen et al. (2014) explained that the increased activity of intestinal alkaline phosphatase in horses is associated with parasitic damage of epithelial cells. Increased EOS count accompanied with higher ALP activity in mares, as found in this study, also may be associated with parasitic damage of epithelial cells.

Significantly higher TBIL concentration in stallions ( $p=0.048$ ) was detected in the present work. Similar gender-related differences were cited by Gupta et al. (2002) and Pađen et al. (2014).

Generally, differences detected in values of observed hematological and biochemical parameters could be due to breed, differences in geographical, physiological, season and climate conditions of sampled horses as well as differences in nutritional factors, management and sample size. Also, differences could be attributed to different reagents, methods and applied instruments.

**In conclusion**, results obtained in the present study showed that gender had much more powerful effects on the investigated parameters in clinically healthy adult Bosnian mountain horses than did the age. Gender had significant effects on six parameters (LYM, EOS, UREA, PHOS, TBIL and AMYL), while age affected two of them

(GLU and UREA). UREA was the only parameter affected by both age and gender. Results published in the present study provide a preliminary baseline-data and could help veterinarians in proper diagnosis, preventive prognosis and therapeutic monitoring.

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