The influence of gender and age on haematological parameters in Herzegovinian donkey

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Introduction

Donkeys are derived from the African wild donkey, although domestication may have occurred several times in regions of its former range, but where it is no longer seen in the present-day distribution. Donkeys are bred for recreational purposes, sport activities, work, milk production and occasionally for meat production (Blench, 2000). The Herzegovinian donkey is a very important animal resource in Bosnia and Herzegovina (B&H). There are currently no information available about the breeds or numbers of animals in B&H. The Herzegovinian donkey is a small donkey. The coat colour varies from grey to brown (Fig. 1). It is estimated that about 100 animals still exist. An endangered animal reserve is currently under construction at Buhovo near Mostar (Kugler et al., 2008).

Determination of physiological parameters can provide valuable information as to the age, sex, nutritional and physiological status of the animal (Osman and Al-Busadah, 2003). It has been shown that differences in haematological profiles by age or gender could be attributed to various processing techniques or applied instruments and nutrition (Stanišić et al., 2015).

The renewed interest for these animals has been demonstrated by studies to establish baseline data of both haematological and biochemical variables in the blood of adult donkeys (Etana et al., 2011; Laus et al., 2015; Stanišić et al., 2015; Zakari et al., 2016). Also, the effects of age and gender on haematological parameters in different donkey breeds have been examined by many authors (Zinkl et al., 1990; Feseha, 1994; French and Patrick, 1995; Folch et al., 1997; Terkawi et al., 2002; Enio et al., 2004; Mori et al., 2004; Gul et al., 2007; Lemma and Moges, 2009; Etana et al., 2011; Laus et al., 2015; Stanišić et al., 2015; Zakari et al., 2016).

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Haematological analysis can provide valuable information about the metabolic status of an individual or a herd, but also about the severity and systemic effects of a disease (Stanišić et al., 2015). The haematological values obtained abroad may not be fully comparable to the local conditions, which are influenced by multiple factors, including breed, environment, and management differences. Some variations also exist in results between laboratories using different reagents, methods and instruments (Gul et al., 2007).

The mean values of certain haematological parameters of Herzegovinian donkey are available in the literature (Rukavina et al., 2016). However, to date there have been no studies to determine the effects of age and gender on the haematological parameters in Herzegovinian donkey. The objective of the present study was to investigate the influence of age and gender on certain haematological parameters in Herzegovinian donkey.

Materials and methods

Blood samples of 30 clinically healthy Herzegovinian donkeys from private farms in Western Herzegovina were collected in August 2010. Based on anamnesis and physical examination, all animals were observed to be clinically healthy. All donkeys were grouped by gender (12 males and 18 females), and were divided into three age groups: young (1-2 years), adult (3-6 years) and old donkeys (7-20 years). Blood samples were taken by external jugular vein puncture in vacuum tubes with ethylenediaminetetraacetic acid (EDTA). The haematocrit (HCT) (%), haemoglobin concentration (Hb) (g/dL), mean corpuscular haemoglobin concentration (MCHC) (g/dL), white blood cells (WBC) ($x10^9$/L), granulocytes (GRAN) ($x10^9$/L), lymphocyte (LYM) ($x10^9$/L) and platelet

Fig. 1. Herzegovinian donkey (Photo: Rukavina D.)

cells (PLT) ($x10^9$/L) were determined using an automated IDEXX QBC VET AutoRead analyser (IDEXX Laboratories, Netherland).

Nonparametric statistical tests were applied in the statistical processing of results using Minitab 17 statistical software. The Mann Whitney test was used to detect the significance of differences between males and females, and the Mood's Median test was applied when comparing three age groups. The Mann Whitney test was also used as a *post hoc* test when the Median test proved to be significant. Resulting p values of less than 0.05 were considered statistically significant. All animals were treated in accordance to the principles of the Internal Ethics Committee for animal welfare and well-being.

Results

Results of the median (range) values of haematological parameters in Herzegovinian donkeys of different gender and age, p values and reference intervals for donkeys are shown in Table 1. Gender significantly affected HCT (p=0.049) and Hb (p=0.046) values and were significantly higher in males than in females. Age significantly affected WBC and LYM counts. Values of WBC and LYM were significantly lower in young than in old donkeys.

Table 1. Median (range) values of haematological parameters in Herzegovinian donkeys of different gender and age, p values and reference intervals for donkeys

Parameters	Gender	Median (range)	p values*	Age (years)	Median (range)	Reference intervals (Gordon and Dewitt, 2010)
HCT (%)	Male	31.00 (23.8-39.4)	0.049	1-2	29.65 (23.8-36.0)	28-47
	Female	27.40 (21.9-39.5)		3-6	24.85 (21.9-39.5)	
				7-20	29.95 (27.7-39.4)	
Hb (g/dL)	Male	11.30 (8.7-14.5)	0.046	1-2	10.85 (8.7-13.2)	9.5-16.5
	Female	10.05 (7.80-13.5)		3-6	9.10 (7.8-13.5)	
				7-20	10.80 (10.2-14.5)	
MCHC (g/dL)	Male	36.60 (35.7-36.8)	0.635	1-2	36.60 (36.1-36.8)	32-36
	Female	36.55 (34.2-36.8)		3-6	36.55 (34.2-36.8)	
				7-20	36.75 (35.7-36.8)	
WBC (x10^9)	Male	11.65 (1.1-13.6)	0.127	1-2	10.30 (0.8-12.0)^b	5.4–15.5
	Female	9.80 (0.8-15.7)		3-6	7.40 (0.9-15.7)^{ab}	
				7-20	11.80 (10.5-13.6)^a	
GRAN (x10^9)	Male	6.10 (0.7-8.4)	0.671	1-2	6.20 (0.5-10.4)	2.2–12.1
	Female	5.50 (0.5-11.7)		3-6	4.65 (0.6-11.7)	
				7-20	6.30 (4.7-8.1)	
LYM (x10^9)	Male	5.55 (0.4-7.7)	0.054	1-2	3.95 (0.3-5.8)^b	1.8–8.6
	Female	3.30 (0.3-7.5)		3-6	1.85 (0.3-7.7)^{ab}	
				7-20	5.90 (3.6-6.6)^a	
PLT (x10^9)	Male	169.0 (8-274)	0.734	1-2	144.00 (8.0-477.0)	160-584
	Female	149.5 (7-477)		3-6	183.50 (9.0-295.0)	
				7-20	120.00 (7.0-211.0)	

* p values less than 0.05 are considered statistically significant

^{ab} median values in column for the same parameter with different superscript are significantly different ($p<0.05$)

Discussion

The present study analysed several haematological parameters in Herzegovinian donkey based on different gender and age. With the exception of the study by Rukavina et al. (2016) on the mean values of certain haematological parameters, there have been no studies on the influence of age and gender on haematological parameters in Herzegovinian donkey. According to that study, most haematological parameters were consistent with the recommended reference ranges for donkeys. The mean value of MCHC was slightly above reference ranges while mean value of PLT was lower than the reference ranges for donkeys in general. In the present study, males had higher mean values of haematological parameters than females. This supports the findings of Etana et al. (2011), who reported that gender effects the blood parameters in many animal species, and values in females are usually

lower than in males. Higher values of haematological values in males are likely the result of more intensive energy metabolism. However, other authors reported no significant gender differences in the haematological parameters of donkeys (French and Patrick, 1995; Folch et al., 1997; Laus et al., 2015).

In this study, gender significantly affected HCT and Hb values. Males had significantly higher values of HCT and Hb ($p = 0.049$; 0.046, respectively). These results were consistent with previous results (Brown and Cross, 1969; Zinkl et al., 1990; Feseha, 1994; Terkawi et al., 2002; Enio et al., 2004). The mean value of HCT in females in this study was lower than the recommended reference intervals for donkeys in general (Gordon and Dewitt, 2010). Also, the HCT and Hb values in male and female Herzegovinian donkeys were lower than values reported in previous studies (Folch et al., 1997; Laus et al., 2015; Stanišić et al., 2015).

In both genders, mean values of MCHC were slightly above the reference intervals for donkeys (Gordon and Dewitt, 2010). According to Zakari et al. (2016), in equines, higher MCHC levels are recorded in summer than in winter or spring. This seasonal influence may be cause of the higher MCHC values presented here, as blood sampling was performed during the summer period. The MCHC values obtained in this study were observed to be similar in both genders. Contrary to our results, Zinkl et al. (1990) reported that female donkeys had higher values of MCHC than male donkeys.

In this study, males had a higher WBC, which counters the findings of Zinkl et al. (1990) and Etana et al. (2011), who found a higher WBC in female donkeys. The LYM count obtained in the present study was higher in males, which is contrary to the higher LYM count in female donkeys reported by Feseha (1994) and Etana et al. (2011).

Female Herzegovinian donkeys had a lower mean PLT value than the reference intervals (Gordon and Dewitt, 2010). In this study, the mean PLT value was higher in males than in females. This is in agreement with the report by Etana et al. (2011), who found that mean PLT values were significantly higher in male working donkeys in Ethiopia than in females.

Analysis of the haematological parameters by age showed slightly different values between young, adult and old donkeys. Values of MCHC showed no differences between the age groups. This supports the findings of Etana et al. (2011), who reported no deviation in MCHC values by age. In this study, young and old donkeys had similar values of HCT and Hb. The lowest values of these parameters was obtained in the adult group. Etana et al. (2011) reported that adult donkeys had higher values of HCT and Hb.

The highest value of LYM and WBC in the present study were observed in old donkeys, while adult donkeys had the lowest values of these parameters. In previous studies (Smith et al., 2002; Aoki and Ishii, 2012), higher LYM and WBC were observed in young donkeys, which is not in accordance to the results presented here. Statistically significant differences between age groups were obtained for WBC and LYM. Young donkeys had significantly lower values of WBC and LYM than old donkeys. Zinkl et al. (1990) reported no effects on LYM count with aging in American donkey. French and Patrick (1995), Terkawi et al. (2002) and Etana et al. (2011) reported higher values of WBC in young donkeys. The total and differential leukocyte counts in healthy donkeys depends on several factors. The most prominent factor is age, which is accompanied by a steady decline of WBC (Folch et al., 1997). The differences between clinical parameters could be result of different breeding, climatic factors, season, management, sample

size, different sampling methods and animal local characteristics (Simenew et al., 2011). However, as in other species, the total and differential leukocyte counts may be altered with age, nutritional status, pregnancy and lactation (Reece, 1997).

In this study, old donkeys were found to have the lowest PLT. This corresponds to Zinkl et al. (1990) and Satue et al. (2009), who reported that age determines a progressive decrease in platelet count. Stanišić et al. (2015) also reported a decreasing platelet count in Balkan donkey with age.

Determination of haematological parameters is important for the further research of the Herzegovinian donkey, as the possible individual and combined influences of different factors such as age and gender of animals could certainly affect the interpretation of the haematological reference values in donkeys. This study is a contribution to the study of haematological parameters in Herzegovinian donkey. The results presented here provide a preliminary baseline that could assist veterinarians in making the proper diagnosis of diseases, preventive prognosis and therapeutic monitoring.

Conclusions

Males had higher mean values of haematological parameters compared to females. In both genders and in all age groups, mean values of MCHC were slightly above the recommended reference intervals for donkeys in general. The mean values of HCT in females and in adult donkeys were lower than the recommended reference intervals, while PLT mean values were lower than the reference intervals for females and in young and old Herzegovinian donkeys. Gender significantly affected HCT and Hb values, which were significantly higher in males. Age significantly affected WBC and LYM count, which were lowest in young donkeys.

Abstract

Several haematological parameters in Herzegovinian donkeys based on gender and age were analysed in this study. The study included 30 clinically healthy donkeys (12 males and 18 females), between one and twenty years of age. Donkeys were grouped in three age classes: young (1-2 years, n=10), adult (3-6 years, n=12) and old donkeys (7-20 years, n=8). A total of seven haematological parameters were analysed. In both genders and in all age groups, the mean values of MCHC were slightly above the recommended reference intervals for donkeys in general. The mean values of HCT in females and in adult donkeys were lower than the reference intervals. The PLT mean values in female, and in young and old Herzegovinian donkeys were lower than the reference intervals. Gender significantly affected HCT and Hb values (p=0.049; p=0.046, respectively), which were significantly higher in males. Age significantly affected WBC and LYM count, which were lowest in young donkeys.

Key words: *gender, age, haematology, donkey*

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Utjecaj spola i dobi na neke hematološke pokazatelje Hercegovačkog magarca

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U ovom smo radu analizirali vrijednosti nekih hematoloških pokazatelja Hercegovačkog magarca na temelju različitosti u odnosu na spol i dob. U istraživanje smo uključili 30 klinički zdravih magaraca oba spola (12 mužjaka i 18 ženki), dobi između jedne i dvadeset godina. Magarce smo podijelili u tri dobne skupine: mladi (1-2 godine, n=10), odrasli (3-6 godina, n=12) i stari magarci (7-20 godina, n=8). Ukupno smo analizirali sedam hematoloških pokazatelja. U oba smo spola i u svim dobnim skupinama uočili da su srednje vrijednosti prosječne koncentracije hemoglobina u krvi bile nešto iznad preporučenih referentnih vrijednosti objavljenih za magarce. U ženki i u odraslih magaraca zapazili smo niže srednje vrijednosti hematokrita u odnosu na preporučene referentne vrijednosti za magarce. Uočili smo da su srednje vrijednosti trombocita u ženki te u mladih i starih Hercegovačkih magaraca bile niže u odnosu na preporučene referentne vrijednosti za magarce. Ustvrdili smo da je spol signifikantno utjecao na vrijednosti hematokrita i koncentracije hemoglobina (p=0,049; p=0,046), a vrijednosti su bile statistički značajno više u mužjaka. Zapazili smo da je dob signifikantno utjecala na vrijednosti leukocita i limfocita. U mladih magaraca smo uočili statistički značajno niže vrijednosti leukocita i limfocita u odnosu na stare magarce.

Ključne riječi: *spol, dob, hematologija, magarac*