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# Physical (Morphometric) and Electrocardiographic Parameters in Balkan Donkey in Serbia

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**Abstract**

Balkan donkey is a native donkey breed that lives in Balkan peninsula and traditionally is reared in Serbia, Macedonia and Montenegro. Since the population has declined severely and only less than 200 males and females are actively breeding the breed status is endangered and it is included in AnGR preservation/conservation program in Serbia. The organized breeding and development of the breed description has started at the beginning of the 21 century and the breed is not fully described yet. This is the first time that that clinical procedures- ECG, have been evaluated in concern with frame and size of Balkan donkey. Our results show that there is a need to expand our investigation to fulfill gap in understanding Balkan donkey physiology and diagnostics.

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The evaluation of morphometric and electrocardiographic parameters in endangered Balkan donkey breed in Serbia was performed and individual data important for clinical evaluation and breeding were estimated in aim to provide initial morphometric and electrocardiographic data for the endangered Balkan donkey breed in Serbia and to assess the ECG procedure in relation to specific anatomy and body proportions of the breed.

***Keywords:*** Balkan donkey; morphometry; electrocardiography.

## 1. Introduction

There are around 44 million donkeys and 163 donkey breeds in the world *[5]*, and their population is declining, especially in Europe *[16, 27]*, while some of the breeds worldwide are facing extinction. The Balkan donkey is indigenous breed that was traditionally reared by sheep and cattle breeders on extensive mountain pastures of Balkan peninsula. The donkey was used as pack animal, carrying goods from mountain pastures to the settlements in the valleys. Due to depopulation of mountain villages and loss of rural tradition the Balkan donkey population has rapidly declined in Serbia from the middle of the 20$^{th}$ century *[14]*, so that the population size is approximated under 500 individuals, while the number of registered breeding males declined to 10 and breeding females to 111 in 2012, facing risk of extinction. The Balkan donkey population was decreasing until the breed was included into Animal Genetic Resources conservation program, in Serbia and the subsidies for the in-situ preservation breeding were introduced. The population status of Balkan donkey in Serbia at present is considered endangered-maintained and the use of the animals is changed into tourist farm attraction and milk producing. The breeding of Balkan donkey was organized at the beginning of the 21$^{st}$ century, when animals assembling historical description of the breed were catalogued and the initiative gave rise to the establishment of Serbian Balkan donkey Studbook. Lack of selective breeding of Balkan donkey is reflected in extensive phenotypic variability, which was revealed during the last couple of decades. During the last decade the data concerning morphometric [16] major hematological and biochemical parameters *[33]* were collected in aim to standardize the breed. Lack of selective breeding of Balkan donkey is reflected in extensive phenotypic variability within the population which was not described until the beginning of the 21$^{st}$ century. Having in mind the small population size and increasing inbreeding, morphological variability can only be attributed to very heterogenous initial population and to very different rearing conditions and very diverse but often insufficient nutrition. Morphometric data determining balance and loading capacities of animals can be used to define functionality measurements: morphological ratios or morphometric indexes that describe the load bearing and carrying capacity including the indices of volume of body cavities, like thoracic cavity and influencing life important and work performance important cardiac and pulmonary function in equines *[30]*. Some studies gave indices that body measurements that determine balance and proportionality of the body can be differently defined in different equine species and in accord to animal working trait *[18]*, thus influencing the position and function of thoracic organs. Unfortunately, although, in human medicine, influence of thoracic shape and intrathoracic volume during development and ageing on cardiopulmonary function and on progression of various diseases is studied in details, the data on influence of shape and size of thoracic cage morphology on development and function of thoracic cavity organs: lungs and heart, are missing in donkeys. However, as the importance of donkeys as genetic resource in Serbia and their use as companion animals and milk producing is increasing, so it became obvious that the breed specific morphological data that can be important for performing

cardiopulmonary clinical procedures and appropriate diagnostic protocols are lacking. Electrocardiography (ECG) in donkey is still poorly developed in comparison to electrocardiography in human, small animal or equine medicine. Limited data is available to ECG itself, so, the electrode positioning and different lead recordings are used upon horse ECG manuals. Weather horse ECG protocol can be appropriately used in cardiac diagnostic in donkeys and the results obtained well interpreted has been questionable, especially in relation with the lack of morphometric characteristics of the thorax in Balkan donkey. Anatomy of the developed thorax greatly influence on pulmonary and cardiac function in donkeys and all mammals, so, the developmental morphometric variability especially the volume of thoracic cavity influence contraction of the heart muscle and ECG findings in these animals. Therefore, the aim of the study was to provide initial morphometric and electrocardiographic data for the endangered Balkan donkey breed in Serbia and to assess the ECG procedure in relation to specific anatomy and body proportions of the breed. So, the evaluation of basic morphometric characteristics in native Balkan donkey and estimation of the specific information on volume and shape of the thorax upon morphological ratios (body frame-BI, proportionality index-PI and Conformation index-CI) in aim to assess the parameters which can influence on the conditions and results obtained by cardiac diagnostic like electrocardiographic evaluation and interpretation of the diagnostic data obtained. In order to evaluate inter-breed similarity within a context of morphometric and ECG parameters, the data obtained for the Balkan donkey were compared with morphometric and ECG parameters of several previously characterized donkey breeds.

## 2. Material and methods

A total of 12 adult Balkan donkeys: 7 females and 5 males, aged between 2 and 15 years, weighting 150-250 kg were included in the study. The females were reared for production of the donkey milk. Animals were identified upon microchip number, coat color, distinctive signs, and age of each animal was determined upon dentition. The study was performed during annual veterinary health assessment in accord to the Animal Health Regulations of Republic of Serbia. Donkeys were in good condition (BCS ranged from 4 to 6) and physically healthy. During the night donkeys were confined in a compartmentalized stable (winter shelter) and free grazing during the day, with addition of common forage diet while in confinement. All animals included in the study were thoroughly clinically examined before the evaluation. No cardiac pathological murmurs were detected on cardiac auscultation.

### *2.1. Morphometric and ECG measurements*

The following morphometric parameters: withers height (WH), body length (BL), chest length (TL), thoracic perimeter (TP), thoracic depth (TD), chest width (CW) and cannon circumference (CC) were estimated by tape measuring in aim to reduce manipulation stress and body weight (BW) was estimated upon the formula (1) by Pejić *[22]*:

$$BW\ (kg) = (TP2 \times BL) / 11877 \qquad (1)$$

Body frame was determined as ratio of WH to BL multiplied by 100, relative body index as ratio of BL to TP multiplied by 100, while Conformation index (CI) was determined upon the formula (2) *[18]:*

$$CI=TP^2/WH \times 0.95 \quad (2)$$

ECG was performed in field conditions with ECG: Schiller Cardiovit C5-100 CEV4.33 with paper speed of 25 mm/s and 10 mm/mV. Each donkey was tested in two intervals of 1 minute. Donkeys were kept on rubber mat to insulate them from the ground. Before each recording, we waited for a period of 5 minutes allowing donkeys to calm down. Alligator clips fixed to the electrocardiographic leads were attached directly to the skin after thorough but careful application of an electrode paste. The ECG electrodes were positioned to obtain a standard base-apex lead from a resting horse, useful to obtain short-term ECG recordings. The same model we used for our measuring, as there is no verified system of positioning ECG electrodes for donkeys *[9]*. The electrode positions described by Einthoven triangle are modified and positioned on the body of the donkey. The right arm electrode – negative (RA) is placed on the right neck of the donkey while the left arm (LA) positive and left leg (LL) electrodes are placed on the left side of the donkey over the apex of the heart. With this electrode configuration, both "Lead I" (RA/LA) and "Lead II" (RA/LL) can be chosen on the ECG recorder to display the base-apex ECG trace. Note that the terminology (LA, RA, LL; lead I, II, III) originates from the Einthoven lead system. *[9, 19]* The ECGs findings were used for estimation of heart rate, amplitude, duration, configuration, segments (PR and ST), and intervals (PP, RR, PR, and QT) in donkeys. The shape and polarity of P and T waves were evaluated. The presence of cardiac dysrhythmias was also evaluated. The classification of dysrhythmias for horse was used *[23]*, because no studies of donkey dysrhythmias are reported in the literature. Measurements of electrocardiographic amplitudes and durations were made manually on three cardiac cycles of each lead, and only in those registers in which outlines were acceptable for their measurement. All procedures involving the donkeys were conducted in accord to good practice and donkey welfare protocol.

### *2.2. Statistical analyses*

The mean values and variation of the morphometric and ECG data obtained were analyzed using Microsoft Excel 16.0 version for Windows 10.

## 3. Results

Our investigation on donkey ECG and physical indices was performed because the demand for veterinary care of Balkan donkey is increasing since their role is changing and more and more donkeys are used as social animals in Serbia. Having in mind the importance of health monitoring and the endangered status of the Balkan donkey population in Serbia we wanted to test weather our ECG protocol, developed for sport horses, can be used in cardiological examinations of small autochthonous donkey. At the same time, we wanted to evaluate the morphometric characteristics because the shape and volume of body cavities can influence physiological function of organs.

### *3.1. Physical characteristics of Balkan donkey*

The following eight body variables were measured: height, body length, chest length, chest circumference, chest depth, chest width, cannon circumference and body weight. The range, average values and standard deviation of each morphometric parameter determined are presented in Table 1.

**Table 1:** Morphometric measurements for the Balkan donkey breed (mean ± standard deviation; range for the whole group

| Parameter | Range (Min -Max) | Mean + SD |
|---|---|---|
| **Withers height (cm)** | 93 - 129 | 109.75±11.34 |
| **Body length (cm)** | 88 - 131 | 115±11. 64 |
| **Chest length (cm)** | 48 - 64 | 54.17±4.84 |
| **Thoracic perimeter (cm)** | 107 - 134 | 118.25±9.64 |
| **Thoracic depth (cm)** | 56 - 80 | 65.92±6.11 |
| **Chest width (cm)** | 22 - 26 | 23.25±2.26 |
| **Cannon circumference (cm)** | 10 - 15 | 12.41±1.98 |
| **Body weight (kg)** | 131- 235 | 174.5±36. 92 |
| **Frame index (cm)** | 90.16 -105.68 | 95.55±4.54 |
| **Chest index (cm)** | 27.14 - 42.86 | 35.5±4.36 |
| **Conformation index (cm)** | 1 09.39 - 140.54 | 121.22±9.33 |

The morphometric data obtained were used to establish the data in relation to development of the body, i.e. body frame, thoracic index and conformation index in Balkan donkey in Serbia. Upon the calculated body indexes (Table1) it was concluded that frame of the donkeys can be slightly rectangular, or almost square, and that thorax is elongated, proportionally deep (sternum reaching the elbow) but narrow in all animal included.

### *3.1. ECG measurements*

The applied ECG procedure gave ECG recordings of good quality. All examined donkeys had a sinus heart rhythm, and no obvious respiratory sinus arrhythmia was recorded. Normal heart rate for Balkan donkey was 55.1 ± 5.21 beats/min as shown in Table 2.

**Table 2:** Duration (s) of the lead II electrocardiographic waves and intervals (mean ± standard deviation; range for the whole group).

| Wawe | Range (Min-Max) | Total number 12-(Mean ± SD) |
|---|---|---|
| **P wawe (sec)** | 0.03 - 1.92 | 0.16 ± 0.28 |
| **RR interval (sec)** | 0.16 - 1.63 | 1.07 ± 0.33 |
| **PR interval (sec)** | 0.17 - 0.92 | 0.26 ± 0.20 |
| **STinterval (sec)** | 0.04 - 0.42 | 0.28 ± 0.05 |
| **QRS segment (sec)** | 0.06 - 0.43 | 0.11 ± 0.10 |
| **T wawe (sec)** | 0.03 - 0.22 | 0.12 ± 0.04 |
| **QT interval (sec)** | 0.06 - 0.57 | 0.44 ± 0.06 |
| **Heart rate (beats/min)** | 47 - 65 | 55.08±5.21 |

P wave was in several cases biphasic and duration was 0.16 ± 0.28 sec. Length of RR interval was 1.07 ± 0.33, and PR interval was 0.26 ± 0.20 sec. ST interval was 0.28 ± 0.05 sec and T wave length was 0.12 ± 0.04sec. The shapes of QRS complexes remained unchanged throughout the experiment. Duration of the QRS complex was 0.11 ± 0.10 sec, and QT interval 0.44 ± 0.06 sec. The heart rate was 55.08 ± 5.21 beats/min. The shape and duration of ECG waves in 12 healthy, adult and relaxed Balkan donkeys obtained in our research did not correspond to ECG in horses *[20]*, which is recommended in equine practice in Serbia.

**4. Discussion**

The morphometric parameters established were used for determination of body indexes that reflect the development of animal, and allow description of the shape, size and volume of body. In comparison of body measurements established with literature data, we found that tested parameters in the Balkan donkey are similar to donkey breeds from neighboring countries and differ from large donkey breeds like Spanish Zamorano-Leones donkey *[16,26]*. Papa and his colleagues [21] evaluated morphometric characteristics of donkey populations in Albania in three regions: lowland, upland and mountain. Their results show similarities in height between the lowland donkey (115,3 ± 8,2cm) and our indigenous breed. In Albania lowland donkey type is defined as a standard type, while highland/mountain type is classified as a miniature Mediterranean type of donkeys. Another study which was done on donkey population in some regions in Bulgaria, shows morphometric congruence between the height of the mountain donkey (112.94±7.21cm) and the results of our study *[28, 31]*. Morphometrical studies in Zamorano-Leones donkey, a Spanish breed, approximated the WH value at 140cm-155cm *[16,26]*, which, compared to our results, shows that the Zamorano-Leones donkey is significantly higher than Balkan donkey in Serbia. Furthermore, Zamorano-Leones has larger thoracic cage and is heavier than Balkan donkey 131-235kg to 245-330kg. The established biometric data reveal characteristic rectangular body frame with narrow chest in Balkan donkey in Serbia, which is similar to already published morphometric data *[28, 33]*. However, the possible influence of the thoracic cage morphological characteristics on ECG findings in Balkan donkey has not been studied previously. As our evaluation is the first measurement of ECG parameters in Balkan donkey, we used standard ECG manual for horses and compared our findings in adult Balkan donkeys with literature data available. We found that tested ECG parameters of Balkan donkey are similar to small donkey breeds from neighboring countries and differ from large donkey breeds like Spanish Zamorano-Leones donkey. All donkeys in our study had a sinus heart rhythm, and no obvious respiratory sinus arrhythmia was recorded. The heart rate and ECG parameters were higher in Balkan donkey compared to Zamorano-Leones and large Iranian donkey, [24]; i.e.; 65 to 52 and 50.26 ± 9.35 beats per minute, respectively *[5]*, while similarity of cardiac parameters with small donkey breeds was obvious. In study of Folch *[7]* maximal HR varied from 62.5 to 93.7 beats/min (mean 72.50 ±7.51), whereas the minimal HR varied from 29.7 to 42.2 beats/min. Differences between large and small breeds could probably be induced by differences in thoracic and heart sizes *[12]*. The described differences were even more pronounced in comparison with *[9,32]* Martina Franca and Amiata donkey breeds which is significantly larger than Balkan donkey, weighting between 250 and 380 kg. In horses, the T wave usually had a positive or biphasic shape *[5,9,19,25]* whereas in the donkey ECG, T wave has a negative shape in majority of cases *[3,8,9,15]*. A negative T wave was recorded in 75 % of animals in our sample (8 of 12 donkeys). According to data available for horses, the change in T wave polarity could be consequential to numerous factors and is most variable wave form in the ECG *[9,10]*. Bifid P

wave was observed in 50% of ECG in all tested donkeys and is described by several authors. *[5,9,10,11]*. P wave analysis showed different data compared to ECG monitoring of adult horses (45%) *[3]* and in Zamorano-Leones donkeys (63%) *[5]*.These findings could be explained by the relationship between vagal tone and training, by age, or by breed differences in donkeys *[9,13,17,23]*. It can also reflect the influence of coronary flow on myocardium which are detected on ECG due to the actual thorax size and shape and thickness of thoracic wall *[1,12]*. Since our donkeys are small and not trained the established P wave findings can only be attributed to morphology, i.e.; shape and size, of the thoracic cage. The duration of the P wave was longer in our donkeys 0.16s than that found by Costa *[4]* 0.108s, Ayala *[2]* 0.105s and shorter than in horses 0.170s *[29]*. These differences might be due to the higher values of heart rate in the Balkan donkey included in our investigation, as they are smaller, compared to that of horses.

## 5. Conclusions

It may be concluded, that morphometric measurements in Balkan donkey are similar to other small donkey breeds from the Mediterranean and to other small donkey breeds worldwide, but differ from large donkey breeds. Having in mind that the body of Balkan donkey can only fully develop in optimal environment and that these animals are bred in traditional extensive conditions it is clear that improved breeding practice in the last decade had influenced the development and growth of these animals thus inducing the huge variation in all observed parameters. Electrocardiogram established in Balkan donkey differs in duration, amplitude and length from those of several larger donkey breeds or from findings in horse breeds. However, the evaluations of the influence of the size and thoracic cage morphology on cardiac function are few in donkeys, but the initial results obtained on different breeds speak that the size of the animal and the volume of thorax influence on cardiac function and ECG finding. These findings justify obtaining values for a specific breed (or similar size breed group) for interpretation of ECG and adapting the diagnostic protocol in accord to morphology of the donkeys. Our findings in concern with the ECG shape, amplitude and duration of the waves, show that extensive investigation on thoracic morphometry and ECG in Balkan donkey are necessary.

## 6. Recommendations

The interesting deviations in shape and duration of the ECG waves were observed in small number of Balkan donkeys included in our preliminary investigation and these findings revealed the need for additional investigation on the influence of thoracic cage morphology in healthy Balkan donkeys both in rest and under physical load in large number of individuals. The further evaluation on ECG diagnostic in our small donkey is also recommended in aim to develop the most appropriate ECG protocol that would consider characteristic morphology of this local breed of small donkey.

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