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Original study

Haematological and biochemical parameters in the blood of an indigenous Croatian breed – Istrian cattle

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Abstract

Haematological and biochemical parameters were examined in the blood of 87 cows, bulls and oxen of the indigenous Croatian breed, Istrian cattle. The sample represents 11.93 % of the total adult population. This breed is classified as highly endangered. The age of tested animals was between 2 and 17 years. Cattle were divided into three groups, animals aged 2 to 6 years (n=19), 7 to 10 years (n=37), and older than 10 years (n=31). Animals were kept in the area of Istria County, mostly in stables though occasionally at pasture. The cows were milked twice daily and gave an average of 1 000 litres of milk per year. Erythrocytes, leukocytes, haemoglobin, haematocrit and mean corpuscular volume were determined. Differential analysis was performed by counting blood cells in the blood smear after Pappenheim staining. Biochemical parameters such as total cholesterol, triglycerides, urea, creatinine, total bilirubin, calcium and inorganic phosphorus were determined. The activity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase was determined by spectrophotometry. These biochemical and haematological parameters of this Istrian breed were within the physiological range, while slight deviations were observed for certain parameters. The greatest deviation from the physiological range was shown in the concentrations of urea and creatinine. These results may contribute to a better understanding of the metabolic profile and haematological indicators for estimating the physiological status of these endangered cattle in Istria, for further investigation and for diagnostic purposes.

Keywords: metabolic profile, hematological profile, Istrian cattle

Abbreviations: CHOD-PAP: cholesterol oxidase – phenol + aminophenazone, GPO-PAP: glycerol-3-phosphate oxidase – phenol + aminophenazone

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Introduction

The Istrian cattle breed is one of three primitive indigenous Croatian cattle breeds that belong to Podolian or grey steppe cattle. Podolian cattle originated directly from European wild cattle (*Bos primigenius Bojanus*, 1827) (Brajkovic *et al.* 1994). Istrian cattle and the Slavonian-SyrmianPodolian cattle have similar genetic characteristics, though there are certain differences between breeds and even inter-breed differences (Keros 2013). The Istrian breed is reported to be genetically closely related to Italian breeds (Astolfi *et al.* 1983), though more similar to the Podolian type (Maremmana and Podolica) than to geographically closer type of breeds (Romagnola, Marchigiana and Chianina), suggesting the possible ancient trade of animals across the Adriatic Sea (Maretto *et al.* 2012). Podolians are characterized by rather slow growth and low milk and meat production. Istrian cattle inhabits the area of the Istrian peninsula in western Croatia. It is a light grey cattle with long horns and a robust shape. It has excellent resistance to disease. Istrian cattle is very modest and does not require much feed. Cattle are usually kept grazing year-round and receive supplemental hay, alfalfa, wheat, and barley straw. Only rarely is their diet enriched with wheat bran. Due to sparse feed, they often graze the leaves of different trees (oak, chestnut, mulberry, fig trees, etc.). Oxen or cows were once used in agriculture to plough the fields, to haul stones for the construction of houses, and their meat and milk served as food for the farmers. However, with modernization, the number of Istrian beef cattle began to drop rapidly. In the 1970s, the Istrian cattle was considered extinct. According to the Annual Report (2012) of the Croatian Agriculture Agency (CAA), there are only 168 breeders holding 39 adult males and 690 adult females of Istrian cattle. This breed has been classified as highly endangered.

The metabolic profile is based on a range of laboratory blood tests and represents the diagnostic starting point for the determination of the nutritional and health status of the animal. The changes in biochemical and haematological constituents are important indicators of the physiological or pathological state of the animal (Ahmad *et al.* 2003).

Early detection of nutrient deficiency or metabolic disease can contribute to successful diagnosis and faster recovery of animal (Radostits *et al.* 2000). Metabolic diseases occur sporadically. In cows with high milk production, metabolic diseases are much more common than in primitive breeds of the same species.

The aim of this study was to examine specific parameters of the state of health, to detect any undesirable changes prior to the appearance of clinical symptoms of these rare animals. Meanwhile, this was a good opportunity to obtain better knowledge of these genetically very old animals, which have remained virtually unchanged through the centuries, and to compare the results with the default values for modern cattle breeds. For this purpose, the metabolic and haematological profiles of the animals were determined.

Material and methods

A total of 87 Istrian adult cattle (n=72 cows, n=15 bulls and oxen) aged from 2 to 17 years were used in the study. This figure represents 11.93 % of the total adult population. Animals were divided into three groups according to age. The first group included cattle aged from 2 to 6 (n=19), the second from 7 to 10 years (n=37) and the third cattle older than 10

years (n=31). The animals were kept mostly in stables with temporary access to pastures. Cattle were fed with hay and grass and in exceptional cases with maize meal or bran. They received fresh water *ad libitum*. A total of 72 cows were milked twice daily, with average milk production of about 1 000 litres milk per year. At the time of blood collection, all individuals were clinically healthy without any clinically visible signs. Blood samples were collected (7.00 to 9.00) from *v. jugularis* during summer period (from June till August). Blood vials for haematological examinations were coated with ethylenediaminetetraacetic acid (EDTA) and those for biochemical studies with sodium heparinate. After blood collection, samples were kept refrigerated and then transported in a cooler at a temperature of +4 °C to the laboratory of the Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb. Erythrocytes, leukocyte, haemoglobin, haematocrit (PCV) and mean corpuscular volume (MCV) values were determined on the haematological apparatus, Coulter type ZF (Model ZF, Coulter Electronics LTD, Harpenden Herts, UK). The differential analysis was performed by counting blood cells in the blood smear after Pappenheim staining.

Biochemical parameters in plasma were determined using the device Technicon RA 1000 (Technicon, Tarrytown, New York, USA). During the determination of enzyme activity, the temperature was held at 37 °C. Total cholesterol was determined according to the CHOD-PAP method, triglycerides to the GPO-PAP method, urea with UV-test modified by Talke and Schubert, creatinine was tested with the Jaffe method, total bilirubin the method of van den Bergh and Muller, calcium method of Gitelman and inorganic phosphorus by the Molibdat method. The activity of aspartate aminotransferase (AST) was determined by spectrophotometry modified by Karmen, the activity of alanine aminotransferase (ALT) modified by Wroblewski and LaDue and the activity of alkaline phosphatase with the method modified by McComb and Bowers. The manufacturer of the test reagents was Randox LTD, Ireland.

The obtained results were analysed by Statistica 6.1 (StatSoft Inc., Tulsa, OK, USA) – statistical calculations of the mean value, standard deviations and standard errors of the mean value. The significant difference among results was examined using the Student's *t*-test, and statistical significance was set at $P<0.05$.

Results

The results of haematological and biochemical parameters are shown in Tables 1 and 2.

The Student *t*-test showed differences ($P<0.05$) in the average number of segmented core neutrophils and lymphocytes between the first and third, the second and third groups of cattle, and the number of eosinophilic granulocytes between the second and third groups of cattle (Table 3). Urea values differed between the first and second, and between the second and third group of cattle, as did values of bilirubin and calcium (Table 4).

Number of segmented core leucocytes was higher in third group than in second group ($P<0.05$), while number of lymphocytes was lower in third in comparison to the second group of Istrian cattle ($P<0.05$). Also, number of eosinophilic granulocytes was significantly higher in third than in the second group ($P<0.05$). Urea concentrations were higher in the first and second than in third group of cattle ($P<0.05$). There were higher concentrations of the bilirubin and calcium in the second in comparison to the third group ($P<0.05$).

Table 1
Haematological parameters in Istrian cattle for three groups according to the age in comparison to The Merck's Veterinary Manual

	Merck's veterinary manual	Group (mean±SEM)		
		1 (n=19)	2 (n=37)	3 (n=31)
Red blood cells (RBC)	5-10×1 0^{12} g/L	6.65 ± 0.79[a]	7.16 ± 1.60[a]	6.65 ± 1.32[a]
Haemoglobin (Hgb)	80-150 g/L	100.89 ± 14.12[a]	107.27 ± 17.39[a]	103.90 ± 22.45[a]
Haematocrit (PCV)	24-46 %	38.62 ± 5.32[a]	42.88 ± 11.34[a]	38.92 ± 8.14[a]
Mean corpuscular volume (MCV)	40-60 fL	57.96 ± 7.94[a]	59.37 ± 7.60[a]	58.47 ± 6.87[a]
White blood cells (WBC)	4-12×10^{9}/L	5.52 ± 1.39[a]	6.21 ± 2.19[a]	5.52 ± 1.83[a]
Neutrophils (band)	0-2 %	0.21 ± 0.92[a]	0[a]	0[a]
Neutrophils (segmented)	15-45 %	29.42 ± 8.61[a]	30.75 ± 6.96[a]	35.67 ± 5.61[a]
Lymphocytes	45-75 %	68.63 ± 10.02[a]	66.59 ± 9.59[a]	60.60 ± 6.32[a]
Monocytes	2-7 %	0[a]	0[a]	0[a]
Eosinophils	2-20 %	2.79 ± 2.75[a]	2.11 ± 3.70[a]	3.73 ± 3.09[b]
Basophils	0-2 %	0[a]	0[a]	0[a]

Values within the same row differ significantly with different exponent ($P<0.05$).

Table 2
Biochemical parameters in Istrian cattle for three groups according to the age in comparison to The Merck's Veterinary Manual

	Merck's Veterinary Manual	Group (mean ± SEM)		
		1 (n=19)	2 (n=37)	3 (n=31)
Total cholesterol, mmol/L	1.6-5.0	4.08 ± 1.42[a]	3.94 ± 1.07[a]	4,05 ± 1.29[a]
Triglycerides, mmol/L	0-0.2	0.43 ± 0.13[a]	0.42 ± 0.16[a]	0,45 ± 0.17[a]
Urea, mmol/L	3.6-8.9	5.89 ± 2.49[a]	4.73 ± 1.89[b]	5.75 ± 1.99[a]
Creatinine, µmol/L	44-194	178.53 ± 19.69[a]	178.27 ± 21.49[a]	173.39 ± 18.29[a]
Total bilirubin, µmol/L	0-27	1.03 ± 0.87[a]	1.43 ± 0.95[a]	1.08 ± 0.61[a]
AST, U/L	60-125	41.58 ± 7.51[a]	38.00 ± 7.11[a]	39.32 ± 8.35[a]
ALT, U/L	6.9-35	15.26 ± 4.36[a]	13.54 ± 3.37[a]	15.00 ± 3.76[a]
AP, U/L	18-153	33.47 ± 14.57[a]	32.97 ± 29.85[a]	33.52 ± 21.55[a]
Ca, mmol/L	2.0-2.8	2.50 ± 0.14[a]	2.43 ± 0.19[a]	2.55 ± 0.35[a]
P, mmol/L	1.8-2.6	1.84 ± 0.54[a]	1.83 ± 0.42[a]	1.94 ± 0.59[a]

ALT: alanine aminotransferase, AP: alkaline phosphatase, AST:aspartate aminotransferase, Values within the same row differ significantly with different exponent ($P<0.05$).

Table 3
Statistical differences of haematological parameters between three investigated Istrian cattle groups (Student *t*-test)

	Group 1 and 2	Group 1 and 3	Group 2 and 3
Red blood cells (RBC)	0.142371	0.995993	0.16118
Haemoglobin (Hgb)	0.155790	1.74743	1.64141
Haematocrit (PCV)	0.065911	0.878543	0.107211
Mean corpuscular volume (MCV)	0.535297	0.825905	0.615381
White blood cells (WBC)	0.161722	0.998701	0.167615
Neutrophils (band)	0.336682	0.330565	0.323985
Neutrophils (segmented)	0.571987	**0.010594**	**0.00237**
Lymphocytes	0.434009	**0.005194**	**0.000266**
Eosinophils	0.444442	0.274715	**0.057415**

Table 4
Differences of biochemical parameters between three investigated Istrian cattle groups (Student *t*-test)

	Group 1 and 2	Group 1 and 3	Group 2 and 3
Total cholesterol	0.699354	0.935271	0.662875
Triglycerides	0.727087	0.632865	0.397026
Urea	0.09347	0.846689	**0.037778**
Creatinine	0.965389	0.37475	0.322343
Total bilirubin	0.12428	0.827716	0.071069
AST	0.1019	0.339705	0.496109
ALT	0.15211	0.8327727	0.10461
AP	0.934295	0.993549	0.932023
Ca	0.139467	0.468138	0.097063
P	0.936343	0.557247	0.389425

ALT: alanine aminotransferase, AP: alkaline phosphatase, AST: aspartate aminotransferase

Discussion

The objective of this study was to determine the haematological and biochemical parameters in the blood of Istrian cattle. To date, there have been few data on specific parameters of the health condition of this highly endangered indigenous Croatian cattle breed (Žubčić *et al*. 2003, Harapin *et al*. 2004). Different values of haematological and biochemical parameters have been reported by various authors, which is understandable due to difference in breeds, keeping and manner of use, feeding and the age of the animals as well as climatic and other factors. Most authors found similar values for erythrocytes, haemoglobin, haematocrit and mean corpuscular volume. According to Schmidl & Forstner (1986) and Pratt (1992), the reference range for lymphocyte values was 40 to 75 %, while the Merck manual lists a range of 45 to 75 % (Smith 1996). For segmented neutrophils, some authors have given reference limits of 20 to 50 % (Smith 1996), while Pratt (1992) gave limits of 15 to 45 %. Some authors

(Sreedhar *et al*. 2013) were founded that during hot season, the indigenous animals suffer from dehydration which results with haemoconcentration, opposite to our findings, inspite blood collection was during summer. According to the results of the present study for the Istrian breed, there were no significant differences between the groups among haematological values: erythrocytes, leukocytes, haemoglobin, haematocrit and mean corpuscular volume, or for biochemical parameters as total cholesterol, triglycerides, urea, creatinine, total bilirubin, calcium and inorganic phosphorus. There were no significant differences of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase enzyme activity. These parameters were in the physiological ranges for Istrian cattle, without significant differences according to age and sex of animals. There were differences in the values of urea, total bilirubin, calcium, segmented neutrophils, lymphocytes and eosinophils between the second and third groups of Istrian cattle according to the Student's *t*-test. Creatinine values were higher than in other publications (Kaneko *et al*. 1997, Kahn 2005). Comparison of the biochemical results to the physiological ranges is complex, as values depend on many factors such as species, breed, sex, age, nutrition, physiological conditions (pregnancy and lactation), illness and seasonal variations (Lumsden *et al*. 1980, Schalm 1984, Tainturier *et al*. 1984, Kaneko *et al*. 1997, Whitaker 1997). The results of biochemical investigations revealed the greatest deviations in concentrations of total serum protein, urea, Na and K which can be affected by different factors (nutrition, health status, stage of lactation and season) (Jezek *et al*. 2013). Mamun *et al*. (2013) were founded a slight increase of calcium, glucose and urea in female compared to the male, and alkaline phosphatase higher in growing cattle, although the variations were not significant ($P>0.05$). Mapiye *et al*. (2012) were observed that most of the indigenous Nguni cattle in South Africa had urea concentration below the reference range which was possibly due to lower intakes of crude proteins on the rangeland pasture. In our study, animals had higher concentration of urea in the blood than in previous study, because cattle were kept mostly in stables with temporary access to the good pastures, as in Sanga cattle from Ghana (Damptey *et al*. 2014) and Thai cattle (Boonprong *et al*. 2007).

The cattle in the present study were on average older than those animals used for production.

The obtained results may represent a contribution to obtaining a better understanding of the metabolic profile and haematological indicators for estimating the physiological status of the endangered Istrian cattle breed, and for diagnostic purposes.

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