Polish Journal of Veterinary Sciences Vol. 18, No. 2 (2015), 439–445

DOI 10.1515/pjvs-2015-0056

Original article

Reference intervals for selected hematological and biochemical variables in Hucul horses

A. Cywińska[1], M. Czopowicz[2], L. Witkowski[2], R. Górecka[3], A. Degórski[1], M. Guzera[1], P. Szczubełek[1], A. Turło[1], A. Schollenberger[1], A. Winnicka[1]

[1] Faculty of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Nowoursynowska 159C, 02-776 Warsaw University of Life Sciences, Poland
[2] Faculty of Veterinary Medicine, Laboratory of Veterinary Epidemiology and Economic, Nowoursynowska 159C, 02-776 Warsaw University of Life Sciences, Poland
[3] Horse Farm, Łosiewice 30, 07-132 Łosiewice, Poland

Abstract

Background: Hucul horses are the unique, genetically distinct breed of Carpathian Mountains. Even though they are recognized as primitive breed, many morphological differences between them and other primitive horses have been reported. Neither hematological nor blood biochemical studies in this breed have been conducted so far.

Objectives: The aim of this study was to establish the reference intervals for basic hematological and selected biochemical variables and to compare them with other breeds.

Material and Methods: Blood samples were collected from 168 Hucul horses and the analyses were performed using routine methods. Mainly nonparametric method was used to establish reference intervals.

Results: The following reference intervals have been established (rounded to two significant digits): RBC: 7.0-13x10^{12}/l; HGB: 106.1-195.8 g/l; HCT: 0.3-0.6 l/l; MCV: 35-50 fl; MCH 11.9-17.1 pg; MCHC: 31.9-34.8 g/dl; WBC: 7.5-22x10^{9}/l, bands: 0-0.5x10^{9}/l; segmented neutrophils: 3.3-10x10^{9}/l; eosinophils: 0-1.1x10^{9}/l; basophils: 0-0.3x10^{9}/l; lymphocytes: 1.9-12x10^{9}/l; monocytes: 0-0.2x10^{9}/l; PLT 95-350x10^{9}/l; MPV 5.2-7.0; ALP: 98-425 U/l; AST: 220-470 U/l; GGT: 9.1-31 U/l; total bilirubin: 6.5-29 µmol/l; CPK: 120-640 U/l; triglycerides: 0.1-0.9 mmol/l; urea: 3.8-11 mmol/l; creatinine: 44 -140 µmol/l; serum amyloid A: 130-5200 µg/l.

Conclusions: Hematological and biochemical variables in Hucul horses were closer to hot-blooded then to cold-blooded and primitive horses or wild equidae. The reference intervals presented in this study pose clinically useful tool for evaluation of blood check-up in Hucul horses.

Key words: Hucul, hematology, blood biochemistry, reference intervals, serum amyloid A

Correspondence to: A. Cywińska, e-mail: anna_cywinska@sggw.pl, tel.: +48 22 593 60 54

Introduction

Hucul breed represents small mountain horses of Carpathian Mountains. It is classified as small-numbered breed and it has been protected as the part of the FAO Program for the Preservation of Animal Genetic Resources since 1979 (Mason 1996, Trandzik et al. 2007, Georgescu et al. 2011). Last years, the renaissance of Hucul horses may be observed and the interest in this breed is increasing, mostly due to their numerous performance values (Purzyc 2007).

Hucul horses are small, characterized by strong and lean constitution, solid body and limb frames, tough hooves, which make them perfectly adapted to the mountain conditions. They are known for their excellent health including disease resistance and tolerance for environmental conditions, high fertility and longevity. They are very neat in character, equable with high vitality, lively temperament and calm disposition (Purzyc 2007, Trandzik et al. 2007, Komosa and Purzyc 2009, Georgescu et al. 2011). Such traits make them skillful for endurance in mountain area, thus they are used as draft horses in forests, riding horses on mountainous routes and pack animals but they are also recommended for hippotherapy. The breed originate from Hucul region in the East Carpathian Mountains, however, the breeding included other regions of Ukraine, Romania, Poland and Slovakia and has been extended also to Czech Republic, Hungary and Austria (Purzyc 2007, Trandzik et al. 2007, Komosa and Purzyc 2009). Hucul horses are used not only near Carpathian Mountains but also in Finland, United Kingdom, Germany, France and even Republic of South Africa (Fornal et al. 2013). At present, the largest population lives in Poland and according to the Polish Horse Breeders Association it counts almost 2500 individuals (PHBA data).

Hucul horses are recognized as primitive breed, as manifested by the phenotype, however, their origin is controversial (Purzyc 2007, Komosa and Purzyc 2009, Georgescu et al. 2011). It is generally believed, that they are the descendant of the Tarpan horse, crossed with different types and breeds including Mongol domestic horses, Przewalski horses as well as Arabians, Lipican, Hafling, Fjord and Nordik breeds (Purzyc 2007, Trandzik et al. 2007). According to the genetic analyses, Hucul breed represents the only autochtonous primitive breed surviving over the centuries in Carpatian Mountains and is totally separate from the other breeds, including pony class: Exmoor, Icelandic Pony, Sorraia, Przewalski Horse, Mongolian Wild Horse, Polish Konik, Shetland Pony and Caspian Pony as well as Arabian and Akhal Teke (Georgescu et al. 2011). The investigation of microsatellite markers has shown that the mean number of alleles per locus was higher than reported in literature for other breeds (Fornal et al. 2013).

In any species, breed traits manifest as diversity in the appearance and disposition, but may also produce differences in blood composition, reflected by variations in hematological and biochemical variables. In horses, several such differences have been reported including higher erythrogram values in hot-blooded horses, low hematocrit in draft horse and pony breeds as well as other minor differences (Krumrych et al. 1993, Smith 2009, Grondin 2010). In the global literature, there are only few reports dealing with Hucul horses, documenting mainly genetic studies (Georgescu et al. 2011, Wnuk et al. 2011, Kusza et al. 2013) and occasionally morphologic research (Purzyc 2007, Purzyc et al. 2011).

Thus, the aim of this study was to extend the characteristics of this breed by defining the reference intervals (RIs) for selected hematological and biochemical variables on the basis of Hucul population in Beskid Niski Mountains.

Materials and Methods

The samples were collected in May 2011, from 168 Hucul horses, of both sexes, aged from 6 months to 23 years, from two main breeding stables in Beskid Niski Mountains (an Eastern part of Carpathian Mountains in Poland). The horses were kept in natural environment, on pasture during the day and in stables at night. Pasture, situated not higher than 600 meters above sea level, gave sufficient nutrition with grain provided once a day. Water and salt were given *ad libitum*. The horses were examined in the morning, when they were in stables. They were handled by their carers to minimalize the stress. The horses were selected on the basis of standard clinical examination and no clinical symptoms of any disease were noted. All horses were dewormed and vaccinated according to standard protocol in the stable. However, neither of these procedures had taken place less than 2 weeks before blood collection. All procedures were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments and approved by Local Ethic Committee. Blood samples were collected by jugular venipuncture into EDTA-3K tubes (Becton Dickinson) for hematological tests and serological tubes (Becton Dickinson) for serum analyses. The tubes were kept in refrigerator (+4°C) and analyzed within 6 hours after collection. The tubes with no anticoagulant were centrifuged at 4380 g for 5 minutes, serum was aspirated, immediately frozen, and stored at -20°C until analyzed. Hematological variables: total number of white

Table 1. Reference intervals (RIs) of hematological variables for Hucul horses.

Variable	Units	Descriptive statistics			Reference Interval		Reference value advisor	
		mean	median	SD	lower limit (CI 90%)	upper limit (CI 90%)	n	method
WBC	$x10^9$/l	13.4	13.3	3.6	7.5 (5.9-8.1)	21.7 (19.4-25.8)	168	NPAR
WBC adults	$x10^9$/l	12.4	12.3	2.9	7.3 (5.9-7.9)	18.1 (16.4-20.2)	135	NPAR
RBC	$x10^{12}$/l	10.1	10.1	1.7	7.0 (6.6-7.3)	13.4 (12.8-14.5)	168	NPAR
HGB	mmol/l	8.9	8.9	1.3	6.6(6.3-7.0)	12.1 (11.7-12.5)	168	NPAR
HCT	l/l	0.43	0.43	0.07	0.31 (0.29-0.33)	0.60 (0.56-0.62)	168	NPAR
PLT	$x10^9$/l	231.7	233	66.2	95.1 (29-129)	352.3 (333-407)	168	NPAR
MCV	fl	42.7	43.0	4.0	35.0 (34.0-36.0)	50.0 (49.0-51.0)	168	NPAR
MCH	fmol	0.89	0.89	0.09	0.74 (0.56-0.76)	1.06 (1.05-1.08)	168	NPAR
MCHC	mmol/l	20.9	21.0	0.7	19.8 (13.7-20.1)	21.6 (21.5-21.8)	168	NPAR
MPV	fl	5.9	5.8	0.5	5.2 (5.0-5.2)	7.0 (6.8-7.6)	168	NPAR
Total neutrophils	$x10^9$/l	6.8	5.8	1.8	3.4 (3.2-3.7)	10.6 (9.5-11.2)	66	NPAR
Bands	$x10^9$/l	0.12	0.12	0.13	0.0 (0-0)	0.54 (0.31-0.67)	66	NPAR
Segments	$x10^9$/l	5.9	5.6	1.8	3.3 (3.1-3.4)	10.3 (10.2-11.0)	66	NPAR
Eosinophils	$x10^9$/l	0.31	0.3	0.24	0.00 (0-0)	1.06 (0.73-1.15)	65	NPAR
Basophils	$x10^9$/l	0.07	0	0.09	0.0 (0-0)	0.35 (0.25-0.44)	66	NPAR
Lymphocytes	$x10^9$/l	6.1	5.8	2.4	1.9 (1.5-2.3)	11.6 (10.5-11.8)	66	NPAR
Monocytes	$x10^9$/l	0.02	0	0.05	0.00 (0-0)	0.20 (0.12-0.31)	66	NPAR

WBC – total number of white blood cells, HCT – hematocrit, HGB – hemoglobin concentration, RBC – number of red blood cells, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, PLT -total number of platelets, MPV – mean platelet volume, NPAR – non-parametric method

Table 2. Reference intervals (RIs) of blood biochemical variables for Hucul horses.

Variable	Units	Descriptive statistics			Reference Interval		Reference value advisor	
		mean	median	SD	lower limit (CI 90%)	upper limit (CI 90%)	n	method
ALP	U/l	220.1	211.1	81.3	98.0 (83.3-120.5)	425.0 (367.1-493.8)	96	NPAR
ALP adults	U/l	191.1	185.5	59.3	89.9 (83.3-117.2)	357.6 (273.1-448.9)	74	NPAR
AST	U/l	325.2	320.5	66.3	222.7 (172.1-231.8)	471.6 (458.2-534.7)	96	NPAR
GGTP	U/l	16.8	16.5	4.8	9.1 (7.8-10.9)	31.0 (25.2-38.9)	96	NPAR
Total bilirubin	µmol/l	15.5	14.2	5.7	6.5 (6.0-8.6)	29.3 (26.5-32.5)	90	NPAR
CPK	U/l	278.8	249.2	131.3	118.2 (112.8-133.5)	644.6 (555.3-677.2)	94	NPAR
TG	mmol/l	0.28	0.21	0.19	0.05 (0.04-0.07)	0.86 (0.70-0.89)	93	NPAR
Urea	mmol/l	7.16	7.3	1.74	3.76 (3.50-4.49)	10.94 (9.57-13.16)	97	NPAR
Creatinine	µmol/l	83.22	81.33	20.07	43.63 (43.32-56.00)	144.00 (115.98-149.4)	93	NPAR
SAA	µg/l	1122.2	802.3	1098.2	129.3 (49.5-266.7)	5153.8 (3622.6-5222.3)	99	NPAR

ALP – alkaline phosphatase, AST – asparagine aminotransferase, GGT – gamma-glutamyl transpeptidase, CPK – creatine phosphokinase, TG – triglicerides, SAA – serum amyloid A, NPAR – non-parametric method, *RUD – robust untransformed data, **RTD – robust transformed data – chosen on account of normal distribution (Anderson-Darling test, p=0.090) and symmetry (test for symmetry, p=0.500) of transformed data, ***STD – standard Box-Cox transformed data – chosen on account of normal distribution (Anderson-Darling test, p=0.058) and lack of symmetry (test for symmetry, p=0.012) of transformed data.

Table 3. Parameters that differ significantly between foals (6 months – 1 year of age) and adult (>1 year of age) Hucul horses.

Parameter	Foals		Adults		Student's t-test p-value
	n	mean (CI 95%)	n	mean (CI 95%)	
WBC [x10^9/l]	33	17.5 (16.3-18.8)	135	12.4 (11.9-12.9)	<0.0001
MCV [fl]	33	37.4 (36.7-38.0)	135	44.0 (43.4-44.5)	<0.0001
PLT [x10^9/l]	33	284.5 (263.7-305.3)	135	218.9 (208.4-229.3)	<0.0001
ALP [U/l]	22	319.0 (290.0-348.1)	74	189.0 (175.7-202.2)	<0.0001
CPK [U/l]	24	182.0 (157.7-206.3)	70	312.0 (280.2-343.7)	<0.0001
TG [mmol/l]	22	0.4 (0.3-0.5)	71	0.2 (0.16-0.22)	<0.0001

WBC – total number of white blood cells, MCV – mean corpuscular volume, PLT – total number of platelets, ALP – alkaline phosphatase, CPK – creatine phosphokinase, TG – triglicerides.

blood cells (WBC), hematocrit (HCT), hemoglobin concentration (HGB), the number of red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total number of platelets (PLT) and mean platelet volume (MPV) were counted with an automated hematology analyzer calibrated for equine species (ABX, Horiba). Differential counts of WBC were obtained from the analyzer and confirmed manually on blood smears by counting 100 cells.

Serum activity of four enzymes – alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), creatine phosphokinase (CPK), as well as serum concentration of five biochemical compounds – total bilirubin, triglicerides (TG), urea, creatinine and serum amyloid A (SAA) were measured.

Clinical biochemistry analyses including ALP, AST, GGT, CPK, total bilirubin, TG, urea, and creatinine were provided with automated clinical biochemistry analyzer (Miura One, ISE. S.r.l., Italy). For all measurements Pointe Scientific (USA) reagents, standards, calibrators and controls were used. SAA concentrations were measured using a double sandwich ELISA (Phase Serum Amyloid A Assay, Tridelta Ltd., Ireland), according to manufacturer protocol.

Blood samples taken from six horses were tested for all morphological and biochemical variables 3 times and the coefficients of variation (CV) were calculated in order to evaluate precision of the tests. For each value CV was below 5%, except for PLT whose CV was between 5 and 10%.

Total number of hematological results from 168 horses was used to calculate RIs for morphological variables, 66 results for leukocyte subpopulations, 97 for biochemical variables and 106 for SAA. Every time cases were removed from the analysis when classified as outliers according to Tukey's rule (Tukey 1977). RIs were calculated using Reference Value Advisor (Geffre et al. 2011). Method of RI calculation depended on the number of available cases and distribution of data points checked with Anderson-Darling test (h=0.05). Briefly, RIs for all variables except for ions were calculated using nonparametric method. Robust Horn or parametric methods were used for ions, with Box-Cox transformation if necessary.

Means of all values were compared between foals (6 months – 1 year-old) and adult horses using Student's t-test for unpaired samples (α=0.05). However, even if significant differences were observed separate RIs were calculated only for adults due to small number of foals (22-33 foals depending on a variable) insufficient to substantiate the decision about partitioning of the sample (CLSI 2010). Linear correlation between leukocyte subpopulations counted by automated and manual methods was evaluated using Pearson's product-moment correlation coefficient on 66 blood samples.

Results

Precise RIs determined for certain hematological and biochemical parameters in Hucul horses are presented in Tables 1 and 2. The variables that differed significantly between foals and adults are shown in Table 3.

Discussion

The results obtained in our study were generally closer to these reported in hot-blooded then in draught and primitive horses (Krumrych et al. 1993, Szarska 1999, Hinchcliff and Geor 2004, Smith 2009, Grondin 2010, Winnicka 2011, Niedzwiedz et al. 2013). However, RIs established for hot-blooded (Szarska 1999, Hinchcliff and Geor 2004, Winnicka 2011), cold-blooded (Smith 2009, Winnicka 2011) and

wild equidae (Walzer 2003) are wide, so the differences are not substantial. In some studies focused on particular breeds narrower ranges were proposed (Geiser and Held 1984, Harvey et al. 1984, Krumrych et al. 1993, Cebulj-Kadunc et al. 2002, Kedzierski and Kowalik 2009, Kedzierski et al. 2009). Most of these reports were provided long time ago using different analytical and statistical methods and the intervals are usually presented as arithmetic mean ± standard deviation. Thus, to investigate breed specific features we compared the values (means ± SD) obtained in our study to those reported for particular breeds rather than general RIs.

From the genetic standpoint Hucul horses are classified separately from all other breeds, including primitive ones (Georgescu et al. 2011). Our results indicate that also hematological variables in Huculs markedly differ from that determined for Polish Konik (referred also as Polish Primitive Horse) (Krumrych et al. 1993). Krumrych et al. (1993) reported that RBC in Polish Konik ($7.1\pm1.1x10^9$/l) was close to that established for American Miniature Horses, Percherons and draught horses, but much lower than RBC determined by us for Huculs ($10.1\pm1.7x10^9$/l). Similarly, HCT and HGB values measured in our study were higher than these observed in Polish Konik (0.43±0.07 *vs.* 0.34±0.04 l/l and 8.9±1.3 *vs.* 8.5±1.7 mmol/l, respectively). Krumrych et al. (1993) interpreted their results as moderate, not low values. Erythrogram results determined in our study were closer to the values proposed for Thoroughbred horses in training (Szarska 1999, Hinchcliff and Geor 2004, Grondin 2010, Winnicka 2011) or to these determined in zebras (Walzer 2003). This fact may be explained by life conditions of Hucul horses, originating from Carpathian Mountains. We do not interpret it as adaptation of individuals to mountain conditions, as the horses examined in our study were kept on pasture not higher than 600 m.a.s.l. It is possible, however that high erythrogram values pose the breed feature in Huculs that increases their tolerance to environmental conditions and predispose them to work in the mountains.

The total numbers of WBC, as well as the numbers of neutrophils determined in our study were high (13.4 ± 3.6 and $5.9\pm1.8x10^9$/l, respectively) when compared to these reported by Krumrych et al. (8.8 ± 2.1 and $4.1\pm1.1x10^9$/l, respectively) but also the ones dealing with hot-blooded horses (including Thoroughbreds and Arabians) (Szarska 1999, Hinchcliff and Geor 2004, Grondin 2010, Winnicka 2011). In the horses, the upper limit of reference intervals are usually established close to $15x10^9$/l, although it is also suggested to interpret the values higher than $14x10^9$/l as moderate leukocytosis (Grondin 2010). On the other hand, the phenomenon known as physiologic leukocytosis, when WBC counts reach $25x10^9$/l, resulting from excitation is frequent in horses, particularly unaccustomed to being handled (Grondin 2010). The high upper limit of RI for WBC observed in our study resulted perhaps from concurrent analysis of data from adults and foals, which had significantly ($p\ll0.001$) higher WBC due to both the age and excitation. When RI was analyzed only for adults the limits were lower. Hucul horses are kept mainly in close-to-natural condition and are not accustomed to blood collection. In this study we minimize the stress by examining horses in the stable where they were handled by their carers. However, in some horses and particularly the foals, the impact of excitation on the results cannot be ignored.

Physiological ranges of leukocyte differential counts presented in the literature differ considerably and the effect of age and physiological status of the horse on the leukocyte subpopulations is emphasized. Thus, our results are generally in line with literature data and no breed-specific features can be proposed. It should also be mentioned that the numbers of neutrophils and lymphocytes computed by the analyzer strongly correlated with that established by manual count ($r=0.78$ and $r=0.88$, respectively, both $p\ll0.001$), but such correlations were not observed in case of eosinophils and monocytes numbers ($r=0.14$, $p=0.259$ and $r=0.06$, $p=0.679$, respectively). Given that neutrophils and lymphocytes are main and the most numerous leukocyte subpopulations, the data obtained from this equipment are accurate and sufficient for clinical application of differential count. However, it is always recommended to perform manual count that allows evaluation of the morphology of blood cells and their precise identification (Barrelet and Ricketts 2002).

Biochemical variables were generally similar to those established for hot-blooded horses (Hinchcliff and Geor 2004, Winnicka 2011), Polish Konik (Kedzierski and Pluta 2013, Niedzwiedz et al. 2013) and wild equidae (Walzer 2003). Only the upper limits of RI for ALP and CPK were surprisingly high. In case of ALP it may be explained by concurrent analysis of data from adults and foals as the latter had significantly ($p<0.001$) higher ALP activity. It is generally known that ALP activity is higher in young animals due to skeletal growth and the values similar to that obtained in our study can be found in literature (Price et al. 1995). When only adults were taken into consideration the upper limit of RI 360 U/l was roughly similar to upper limits established by other authors (Winnicka 2011, Niedzwiedz et al. 2013).

The upper limit of RI for CPK activity determined in our study was higher than commonly accepted in

equine practice (Szarska 1999, Hinchcliff and Geor 2004, Kedzierski et al. 2009, Smith 2009, Kedzierski 2011, Winnicka 2011, Kedzierski and Pluta 2013). Several factors reflecting the status of skeletal muscles are known to result in an increase in CPK activity. Low-grade chronic muscle damage and short term reversible effect of strenuous exercise seem the most important (Szarska 1999, Hinchcliff and Geor 2004, Winnicka 2011, Niedzwiedz et al. 2013). The horses involved in our study did not exercise. In case of muscle damage, regardless the cause, CPK activities reach much higher values than determined in our study (Szarska 1999, Knoepfli 2002, Smith 2009). The other cause of higher CPK activity is the contamination of needle with tissue during blood collection (Meyer 2004), however, it is not likely in the horses examined in our study. It is generally accepted that CPK activity is related to the muscle mass. This has been confirmed in our study by lower values in the foals than in adults. Thus, it can be postulated that high CPK activity in Hucul horses reflects the relatively large muscle mass in this breed.

SAA is usually not included in routine blood analysis, however, it is known as good indicator of inflammation. Moreover, it has been reported as useful in the evaluation of herd health status (Petersen et al. 2004). Commonly accepted RI for SAA is very wide (0-20 mg/l) (Hinchcliff and Geor 2004), but the values reported in horses well trained for endurance competitions did not exceed 1 mg/l (Cywinska et al. 2010). The levels detected in healthy, but not regularly trained horses were below 7 mg/l (Hulten et al. 1999) and similar results were obtained also in our study.

In conclusion, this is the first study providing RIs for blood variables for Hucul horses. The results have shown that basic hematological variables in Hucul horses are closer to hot-blooded horses than to Polish Konik. RIs are quite wide due to various age and physiological status of horses. Ideally, it is recommended to establish a set of normal values for each individual. In this way early and subtle change may be detected (Barrelet and Ricketts 2002, Meyer 2004, Kedzierski and Pluta 2013). It is useful and practiced in case of performance horses but not in Hucul stables where veterinary examination is much less frequent. Thus, breed-specific RIs seem to be useful in veterinary practice.

Acknowledgments

The study was partially supported by a grant from National Science Centre on the basis of the decision No. DEC-2011/03/B/NZ6/04682.

The authors would like to thank Mr. Sławomir Bogusz (Horiba, Polska) for providing the access to Horiba equipment.

References

Barrelet A, Ricketts S (**2002**) Haematology and blood biochemistry in the horse: a guide to interpretation. In Practice 24: 318-327.

Cebulj-Kadunc N, Bozic M, Kosec M,Cestnik V (**2002**) The influence of age and gender on haematological parameters in Lipizzan horses. J Vet Med A Physiol Pathol Clin Med 49: 217-221.

Clinical and Laboratory Standards Institute (**2010**) Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed., Wayne, USA.

Cywinska A, Gorecka R, Szarska E, Witkowski L, Dziekan P, Schollenberger A (**2010**) Serum amyloid A level as a potential indicator of the status of endurance horses. Equine Vet J Suppl: 23-27.

Fornal A, Radko A, Piestrzyńska-Kajtoch A (**2013**) Genetic polymorphism of Hucul horse population based on 17 microsatellite loci. Acta Biochim Pol 60: 761-765.

Geffre A, Concordet D, Braun JP,Trumel C (**2011**) Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 40: 107-112.

Geiser DG, Goble DO, Held JP (**1984**) Normal hematology and serology of the Clydesdale draft horse. Equine pract 6: 7-9.

Georgescu SE, Manea MA, Dudu A, Costache M (**2011**) Phylogenetic relationships of the Hucul horse from Romania inferred from mitochondrial D-loop variation. Genet Mol Res 10: 4104-4113.

Grondin TD, Dewitt SF (**2010**) Normal hematology of the horse and donkey. In: Weiss DJ, Wardrop KJ (eds) Schalm's Veterinary hematology. 6th ed., Wiley-Blackwell, Iowa, pp 821-829.

Harvey RB, Hambright MB, Rowe LD (**1984**) Clinical biochemical and hematologic values of the American Miniature Horse: reference values. Am J Vet Res 45: 987-990.

Hinchcliff KK, A. Geor R (**2004**) Equine sports medicine and surgery. 1st ed. Oxford, Saunders, pp 1294-1301.

Hulten C, Tulamo RM, Suominen MM, Burvall K, Marhaug G, Forsberg M (**1999**) A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA) – a clinically useful inflammatory marker in the horse. Vet Immunol Immunopathol 68: 267-281.

Kedzierski W (**2011**) Correlation of plasma creatine kinase activity and glucose level in exercised Purebred Arabian horses. Med Weter 67: 541-544.

Kedzierski W, Bergero D, Assenza A (**2009**) Trends of Hematological and Biochemical Values in the Blood of Young Race Horses during Standardized Field Exercise Tests. Acta Veterinaria (Beograd) 59: 457-466.

Kedzierski W, Kowalik S (**2009**) Leptin and Ghrelin and the Indices of Lipid Metabolism as Related to Sex Steroid Hormones in Trotters. J Equine Vet Sci 29: 17-23.

Kedzierski W, Pluta M (**2013**) The welfare of young Polish Konik horses subjected to agricultural workload. J Appl Anim Welf Sci 16: 35-46.

Knoepfli AB (**2002**) Exertional rhabdomyolysis in a 4-year-old standardbred filly. Can Vet J 43: 293-295.

Komosa M, Purzyc H (**2009**) Konik and Hucul horses: a comparative study of exterior measurements. J Anim Sci 87: 2245-2254.

Krumrych W, Wisniewski E, Danek J (**1993**) Haematological parameters of the Polish Primitive Horses. Arch Vet Pol 33: 205-216.

Kusza S, Priskin K, Ivankovic A, Jędrzejewska B, Podgórski T, Javor A, Mihok S (**2013**) Genetic characterization and population bottleneck in the Hucul horse based on microsatellite and mitochondrial data. Biol J Linn Soc 109: 54-65.

Mason I (**1996**) A World Dictionary of Livestock Breeds, Types and Varieties. 4th ed, C.A.B International, p 273.

Meyer DH, Harvey JW (**2004**) Veterinary laboratory medicine: interpretation and diagnosis. 3rd ed., Saunders, Philadelphia, pp 3-12.

Niedzwiedz A, Jaworski Z, Filipowski H, Zawadzki M, Wrzosek M, Sluzewska-Niedzwiedz M, Nicpon J (**2013**) Serum biochemical reference intervals for the Polish Konik horse (Equus cabalus gmelini Ant.). Vet Clin Pathol 42: 66-69.

Petersen HH, Nielsen JP, Heegaard PM (**2004**) Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res 35: 163-187.

Polish Horse Breeders Association, Breeding Statistics. http://pzhk.pl/hodowla/statystyka-hodowlana/

Price JS, Jackson B, Eastell R, Goodship AE, Blumsohn A, Wright I, Stoneham S, Lanyon LE, Russell RG (**1995**) Age related changes in biochemical markers of bone metabolism in horses. Equine Vet J 27: 201-207.

Purzyc H (**2007**) A general characteristic of Hucul horses. Acta Sci Pol – Medicina Veterinaria 6: 25-31.

Purzyc H, Kobrynczuk F, Bojarski J (**2011**) Sexual dimorphism in Hucul horses using discriminant analysis. Animal 5: 506-511.

Smith B (**2009**) Normal Values for Erythron Data, Normal Values for Leukogram Data In: Large animal internal medicine, 4th ed., Mosby Inc. p 1822.

Szarska E (**1999**) Badania laboratoryjne w treningu koni. Agencja Reklamowa „Crex", Warszawa.

Trandzik JZ, R. Jakabova, D. Buleca, J. Massanyi, P. Hasko, M. Kozlik, P (**2007**) Genetic diversity of Hucul horse, based on microsatellite data in Slovak Republic. http://www.raumberg-gumpenstein.at/c/index.php?option=com_docman&task=doc_view&gid=2344&Itemid=100014

Tukey J (**1977**) Exploratory Data Analysis Pearson, 1st ed., Prinston University, USA, pp 120-127.

Walzer C (**2003**) Equidae. In: Fowler ME, Miller RE (eds) Zoo and wild animal medicine. 5th ed., Saunders, St. Louis, pp 578-586.

Winnicka A (**2011**) Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. 5th ed., Wydawnictwo SGGW, Warszawa, pp 17-74.

Wnuk M, Bugno-Poniewierska M, Lewinska A, Oklejewicz B, Zabek T, Bartosz G, Slota E (**2011**) Age-related changes in genomic stability of horses. Mech Ageing Dev 132: 257-268.