Determination of haematological and biochemical parameters of Przewalski horses (*Equus przewalski*) kept by the Prague Zoo

J. Tomenendalova[1], R. Vodicka[2], I. Uhrikova[1], J. Doubek[1]

[1]Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]The Prague Zoological Garden, Prague, Czech Republic

ABSTRACT: The objective of this study was to determine ranges of haematological and biochemical parameters of Przewalski horses (*Equus przewalski*) kept in the Czech Republic, to compare the results with data reported for the same species as well as for domestic horses (*Equus caballus*), and to evaluate differences between both genders and age groups. Stallions showed significantly higher counts of red blood cells and bands, higher levels of total bilirubin and magnesium, and a higher activity of GGT. Higher concentrations of cholesterol, vitamin A and sodium were found in mares. Young animals were characterised by a lower count of eosinophilic granulocytes, a higher activity of ALP and higher concentrations of calcium, inorganic phosphorus, sodium and creatinine. Other differences were not significant.

Keywords: blood parameters; reference ranges; in captivity

Reference ranges of physiological parameters can be useful for the evaluation of the state of health in specimens of the species as well as diagnostics and prevention of diseases. There are numerous studies on reference values in the domestic horse, both the species and individual breeds.

On the other hand, reference ranges of haematological and biochemical values of Przewalski horses kept in captivity are nearly completely lacking. Data are often based on low numbers of samples quite insufficient for the determination of reference ranges. Przewalski horses are kept in individual parks and zoos under various conditions, climatic regions and their fitness and state of health may differ. Blood parameters can also be influenced by the sampling procedure, anaesthesia, immobilisation, handling and stress. It is, therefore, difficult to compare results of individual patients with published data.

The objective of this study was to determine ranges of haematological and biochemical parameters of Przewalski horses kept by the Prague Zoo (Czech Republic) and to compare the results with data reported previously. As only limited data exist on haematological and biochemical parameters of this equine species, patients have often been evaluated using reference ranges of the domestic horses. The results of this study have, therefore, been compared with reference values of domestic horses. Effects of age and gender on these parameters have also been evaluated.

MATERIAL AND METHODS

Animals. A total of 24 clinically healthy Przewalski horses (17 mares and seven stallions aged from one to 17 years) were included in this study. Groups of one- to five-year-old horses and horses older than five years included nine and 16 specimens, respectively (one mare was blood sampled when younger as well as older than five years). The horses came from three regions of the Czech Republic (i.e., Slatinany, Dobrejov, Prague) managed by Prague Zoo.

Nutrition: The summer ration is based on green feeds. Winter feeds are composed of hay supplemented with grain, apples and vegetables, or pastry.

Collection of samples. Blood samples were collected from 1997 to 2002. Some horses were sampled on several occasions.

Horses were fasted for 24 h prior to blood collection, while water was provided without limitation. In order to collect samples horses were immobilised using a combination of detomidine (Cepesedan 10 mg/ml inj., detomidini hydrochloridum, CP – Pharma GmbH, Germany), and Large Animal Immobilon (2.45 mg etorphine hydrochloride + 10 mg acepromazine maleate, Novartis Animal Health UK Ltd, UK). Blood was then collected within 15 min from the jugular vein.

Blood was collected into Vacuette® test-tubes (containing K_3EDTA) for haematology and Z Serum Clot Activator for biochemistry (both produced by Greiner Bio-One, Austria). Haematological samples were kept at 4 °C and examined within 48 h of collection. Blood for biochemistry was allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 min. The serum obtained was kept in sterile vials at –19 °C and examined within three months of collection.

Laboratory examination. Red and white blood cell counts were obtained using the Bürker counting chamber method. Red blood cells were stained with Hayem's solution (Zkoumadlo Hayem, Penta, Chrudim, Czech Republic). A total of 25 µl of blood were added to vials containing 4975 µl of Hayem's solution (i.e., 200× dilution). Red blood cells were then counted in the Bürker chamber using a light microscope. White blood cells were counted using Türk's solution (Zkoumadlo Türk, Penta, Chrudim, Czech Republic). A total of 25 µl of blood were added into vials containing 475 µl of Türk's solution (i.e., 20× dilution). White blood cell differential counts were obtained from blood smears stained with Diff-Quick using an oil immersion objective and 1000x magnification.

Haematocrit was obtained following blood centrifugation in microcapillaries, while haemoglobin concentrations were measured using the Bio-La-Test Sono Hb (Pliva Lachema, Czech Republic). Haematocrit and red blood cell counts, haemoglobin and red blood cell counts, and haemoglobin and haematocrit were employed to determine MCV, MCH, and MCHC, respectively.

Biochemical parameters were determined photometrically. Concentrations of urea, total bilirubin and the activity of creatinkinase (CK) were determined using Pliva Lachema (Czech Republic) tests. Concentrations of glucose, total protein and cholesterol and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyltransferase (GGT) were measured using BioVendor (Czech Republic) tests. The concentration of albumin was determined using Albumin liquicolor (Human, Germany). DiaLab (Czech Republic) tests were used for the determination of concentrations of creatinine, inorganic phosphorus and chlorine.

Concentrations of minerals such as sodium, potassium, calcium, magnesium, zinc and copper were determined using a H 1550 analyser (Hilger, UK) and the F-AAS method. Vitamin A and E concentrations were obtained through fluorescence spectrophotometry.

Statistical examination. Outliers were excluded using Grubbs' test (Lumsden and Mullen 1978). Results are provided as the mean ± standard deviation and median. Student's *t*-test was employed to compare the measured parameters with published data. When comparing age groups and both genders the datasets were first processed using the *F*-test for homogeneity of variances and then using the *t*-test. Values of $P < 0.05$ and $P < 0.01$ were considered statistically significant and highly significant and designated * and ** in tables, respectively, for all tests. MS Excel and RExcel was used to perform statistical analyses.

RESULTS

Haematological parameters

Minimum and maximum values, medians, means and standard deviations of haematological parameters are shown in Table 1. Table 2 presents acomparison of females and males (means and standard deviations as well as minimum and maximum values); statistical significance was evaluated using the *t*-test. Comparison of haematological parameters of one- to-five-year-old horses and horses older than five years (means and standard deviations as well as minimum and maximum values) is given in Table 3, while Table 4 compares haematological parameters based on all specimens examined (without considering the age and gender) with published data (Lumsden et al. 1980; Kuttner and Wiesner 1987; Baronetzky-Mercier 1992).

Biochemical parameters

Minimum and maximum values, medians, means and standard deviations of biochemical parameters are shown in Table 5. Table 6 presents a comparison of mares and stallions (means and standard deviations as well as minimum and maximum values, *t*-test). A comparison of biochemical parameters of one- to five-year-old horses and horses older than five years (means and standard deviations as well as minimum and maximum values, *t*-test) is given in Table 7. The results of the present study are compared with published data (Kuttner and Wiesner 1987; Baronetzky-Mercier 1992) in Table 8. Because of the low number of specimens, differences were not statistically evaluated. Table 9 provides a comparison of concentrations of zinc and copper with values reported for the domestic horse (Grace et al. 1999; Ayetkin et al. 2010; Yur et al., 2008).

DISCUSSION

Haematology

Gender differences in haematological parameters were not considerable. Males were characterised by significantly higher red blood cell counts ($P < 0.05$) as well as immature neutrophilic granulocytes ($P < 0.05$) than females. Stallions also showed higher values of haemoglobin ($P = 0.77$), white blood cells ($P = 0.74$) and monocytes ($P = 0.56$), but without statistical significance. In contrast, the MCV index was lower ($P = 0.65$). As there are no relevant references comparing haematological parameters between genders in the Przewalski horses, we compared our results with reports on other equids. Similar differences as in the Przewalski horses were reported for feral domestic horses (*E. caballus*) by Plotka et al. (1988): stallions had statistically higher red blood cell counts, haematocrit and haemoglobin concentrations when compared with mares. Contrariwise, Lumsden et al. (1980) and Folch et al. (1997) did not find statistically significant differences between stallions and mares in domestic horses and Catalonian donkeys (*E. asinus*), respectively.

The results of the present study show only small differences between age groups. The lower eosinophilic granulocyte count in the younger horses was statistically significant ($P < 0.05$). The red blood cell count was also lower ($P = 0.055$), while the MCH index was higher ($P = 0.055$). A study on the Spiti horses from India showed an opposite trend (Gupta et al., 2005); i.e., levels of haemoglobin, haematocrit, red and white blood cells decreased with age, but without statistical significance. According to Folch et al. (1997) young donkeys had significantly lower values of MCV and MCH as well as higher counts of leukocytes, immature neutrophils, segmented neutrophils and eosinophils than older animals.

Comparison between our data and references on the Przewalski and domestic horses yielded inter-

Table 1. Range of haematological parameters. All specimens evaluated consideration of age and gender

	$\bar{x}$ ± SD	Median	Min–max	*n*
RBC ($\times 10^{12}$/l)	6.6 ± 1.2	6.32	4.92–9.82	21/35
Haematocrit (l/l)	0.41 ± 0.05	0.42	0.3–0.52	23/37
Haemoglobin (g/l)	132.07 ± 22.14	134.75	91.55–187.15	17/32
MCV (fl)	63.61 ± 10.2	63.2	40.15–83.61	20/35
MCH (pg)	19.6 ± 3.7	19.55	12.43–29.61	16/30
MCHC (g/l)	314.44 ± 52.16	312.06	179.51–445.6	17/32
WBC ($\times 10^9$/l)	6.68 ± 1.55	6.15	4.3–10.2	21/36
Bands ($\times 10^9$/l)	0.26 ± 0.14	0.28	0–0.73	21/35
Segments ($\times 10^9$/l)	3.08 ± 1.05	2.85	1.57–7.24	21/36
Lymphocytes ($\times 10^9$/l)	2.92 ± 0.80	2.86	1.68–4.84	21/36
Monocytes ($\times 10^9$/l)	0.34 ± 0.24	0.28	0.05–0.98	21/36
Eosinophils ($\times 10^9$/l)	0.09 ± 0.16	0	0–0.62	21/36

Minimum and maximum values, *n* = number of horses/number of samples

Table 3. Comparison of haematological parameters of one- to five-year-old horses and horses older than five years

	One- to five-year-old horses			Horses older than five years			Statistical evaluation	
	$\bar{x}$ ± SD	min–max	*n*	$\bar{x}$ ± SD	min–max	*n*	*F*-test	*t*- test
RBC (×10^{12}/l)	6.09 ± 0.83	4.92–7.34	7/8	6.95 ± 1.42	5–9.92	16/29	0.144	0.055
Haematocrit (l/l)	0.4 ± 0.06	0.3–0.48	7/8	0.42 ± 0.05	0.33–0.52	16/29	0.357	0.189
Haemoglobin (g/l)	141.7 ± 13.66	126.1–159.2	4/5	130.3 ± 23.12	91.55–187.2	14/27	0.315	0.149
MCV (fl)	65.92 ± 6.83	55.35–72.2	7/8	61.84 ± 11.61	38.31–83.61	16/29	0.150	0.176
MCH (pg)	21.82 ± 1.82	18.73–23.41	4/5	18.83 ± 3.95	12.01–29.61	14/27	0.141	0.055
MCHC (g/l)	328.01 ± 22.21	303.3–362.45	4/5	311.9 ± 55.92	179.5–445.6	14/27	0.085	0.268
WBC (×10^{9}/l)	7.05 ± 1.98	4.8–10	7/8	6.58 ± 1.43	4.3–10.2	15/28	0.215	0.228
Bands (×10^{9}/l)	0.25 ± 0.21	0.09–0.73	7/8	0.26 ± 0.11	0–0.44	15/27	0.016	0.473
Segments (×10^{9}/l)	3.37 ± 0.97	1.87–4.59	7/8	2.99 ± 1.07	1.57–7.24	15/28	0.839	0.189
Lymphocytes (×10^{9}/l)	3.11 ± 0.94	2.24–4.6	7/8	2.86 ± 0.76	1.68–4.84	15/28	0.409	0.221
Monocytes (×10^{9}/l)	0.30 ± 0.19	0.17–0.7	7/8	0.35 ± 0.25	0.05–0.98	15/28	0.407	0.276
Eosinophils (×10^{9}/l)	0.02 ± 0.04	0–0.12	7/8	0.09 ± 0.14	0–0.41	15/27	0.004	0.026*

Minimum and maximum values, *n* = number of horses/number of samples

Table 2. Comparison of haematological parameters of mares and stallions

	♀			♂			Statistical evaluation	
	$\bar{x}$ ± SD	min–max	*n*	$\bar{x}$ ± SD	min–max	*n*	*F*-test	*t*-test
RBC (×10^{12}/l)	6.26 ± 0.8	5.00–8.22	14/22	7.19 ± 1.54	4.92–9.82	7/13	0.008**	0.031*
Haematocrit (l/l)	0.41 ± 0.05	0.30–0.52	15/24	0.42 ± 0.05	0.34–0.51	7/13	0.535	0.242
Haemoglobin (g/l)	128.0 ± 20.20	98.20–160.70	13/21	139.84 ± 24.46	91.55–187.15	5/11	0.453	0.077
MCV (fl)	65.63 ± 9.50	40.15–82.40	14/22	60.20 ± 10.80	44.64–83.61	7/13	0.586	0.065
MCH (pg)	20.01 ± 3.21	12.43–26.16	11/19	18.90 ± 4.51	15.01–29.61	5/11	0.201	0.220
MCHC (g/l)	309.28 ± 45.67	229.09–386.11	12/21	324.30 ± 64.04	179.51–445.60	5/11	0.190	0.224
WBC (×10^{9}/l)	6.42 ± 1.51	4.30–10.00	15/24	7.22 ± 1.56	4.90–10.20	6/12	0.849	0.074
Bands (×10^{9}/l)	0.23 ± 0.16	0.00–0.73	15/24	0.33 ± 0.07	0.20–0.44	6/12	0.018*	0.019*
Segments (×10^{9}/l)	2.96 ± 0.76	1.87–4.59	15/24	3.31 ± 1.48	1.57–7.24	6/13	0.007**	0.231
Lymphocytes (×10^{9}/l)	2.86 ± 0.76	1.68–4.60	15/24	3.02 ± 0.90	2.04–4.84	6/13	0.476	0.299
Monocytes (×10^{9}/l)	0.30 ± 0.21	0.05–0.98	15/24	0.43 ± 0.27	0.10–0.98	6/13	0.321	0.056
Eosinophils (×10^{9}/l)	0.06 ± 0.11	0.00–0.39	15/24	0.16 ± 0.22	0.00–0.62	6/11	0.007**	0.102

Minimum and maximum values, *n* = number of horses/number of samples, *$P < 0.05$, **$P < 0.01$

Table 4. Comparison of haematological parameters based on all specimens examined (without consideration of age and gender) with published data concerning both the Przewalski and domestic horses

	E.p. ZOO Prague (n = 30–37)	E.p. Kuttner (n = 20)	E.p. ISIS (n = 39–78)	E.p. Baronetzky-Mercier (n = 4)	E.p. Hawkey (n = 4)	E.c. Lumsden (n = 44–50)
RBC ($\times 10^{12}$/l)	6.6 ± 1.2	8.9 ± 0.9	N	N	8.6 (8.4–10.8)	8.8 ± 0.95
Haematocrit (l/l)	0.41 ± 0.05	43.7 ± 3.7	0.42 ± 0.07	N	0.47 (0.38–0.50)	0.40 ± 0.04
Haemoglobin (g/l)	132.07 ± 22.14	155 ± 1.7	154 ± 2	N	163 (14.3–20)	146 ± 16.5
MCV (fl)	63.61 ± 10.2	51 ± 8	35.9 ± 20	N	55.1 (45–66)	45 ± 2.4
MCH (pg)	19.6 ± 3.7	18 ± 2	13 ± 7.2	N	19.2 (17.0–21.6)	16.6 ± 0.9
MCHC (g/l)	314.44 ± 52.16	350 ± 20	364 ± 24	N	352 (320–360)	372 ± 12
WBC ($\times 10^9$/l)	6.68 ± 1.55	8.26 ± 1.68	8.3 ± 2.5	7.5 (5.1–9.2)	9.6 (8.4–10.8)	7.45 ± 1.28
Bands ($\times 10^9$/l)	0.26 ± 0.14	N	N	N	N	0.06 ± 0.1
Segments ($\times 10^9$/l)	3.08 ± 1.05	5.18 ± 1.44	N	N	N	3.75 ± 0.88
Lymphocytes ($\times 10^9$/l)	2.92 ± 0.80	2.8 ± 0.81	N	N	N	3.10 ± 0.75
Monocytes ($\times 10^9$/l)	0.34 ± 0.24	N	N	N	N	0.2 ± 0.1
Eosinophils ($\times 10^9$/l)	0.09 ± 0.16	N	N	N	N	0.2 ± 0.2

E.p. = *Equus przewalski*, E.c. = *Equus caballus*, N = not available

esting results. The examined specimens showed lower levels of haematological parameters such as red blood cells, haematocrit, haemoglobin, MCHC as well as white blood cells when compared with both domestic horses (Lumsden et al. 1980) and Przewalski horses (ISIS 1987; Kuttner and Wiesner 1987). On the other hand, MCV and MCH values were higher. There were only slight differences between our ranges of parameters and reference values in the domestic horses. The lower counts of blood elements in comparison with the domestic horses can be caused by many factors. The collection of blood from animals under general anaesthesia and the higher stress in animals not accustomed to daily handling can be one of these factors. Kuttner and Wiesner (1987) reported a significant drop in haemoglobin, haematocrit and red blood cell count in the course of anaesthesia using Immobilon due to red blood cell sequestration in the spleen. This mechanism may also be responsible for the lower white blood cell count. Other factors can include subclinical anaemia or different procedures of counting blood elements (manual counts versus automated analysers). Reference ranges for leukocytes (total and differential counts) are wider in the present study than in reports on the domestic horse, but without significant differences. The median value of the white blood cell count was lower than reported by other authors, similar to the absolute count of segmented neutrophils. The count of immature neutrophilic granulocytes was significantly higher than reported by Lumsden et al. (1980) for horses. No differences were observed in the absolute lymphocyte count.

Biochemistry

Total bilirubin and magnesium concentrations were higher in stallions than mares ($P < 0.05$). Males showed a higher activity of GGT ($P < 0.01$), and females of LDH ($P < 0.01$). Mares were characterised by higher concentrations of cholesterol ($P < 0.01$), vitamin A ($P < 0.05$) and vitamin E (non-specific) and sodium ($P < 0.05$). Asadi et al. (2006), on the other hand, reported a higher cholesterol concentration in stallions. Our results correspond with those of Blakley and Bell (1994) who reported higher concentrations of vitamins in mares than stallions.

As expected, younger specimens had a significantly higher activity of ALP ($P < 0.05$), inorganic phosphorus ($P < 0.01$) and calcium ($P < 0.05$) concentrations than adult horses, a phenomenon associated with growth and higher bone metabolism. The same results were obtained by other authors (Lumsden et al. 1980; Gossett and French 1984; Price et al. 1995; Gupta et al. 2005). Concentrations

Table 5. Ranges of biochemical parameters. All specimens evaluated without consideration of age and gender

	$\bar{x}$ ± SD	Median	Min–max	*n*
Total protein (g/l)	64.1 ± 7.2	65.5	48.9–81	24/41
Albumin (g/l)	32 ± 3.5	32.1	24.5–38.5	24/41
Globulin (g/l)	31.9 ± 5.6	31.25	20.5–43.7	21/36
A/G	1 ± 0.2	0.99	0.57–1.42	21/37
Glucose (mmol/l)	8.68 ± 2.77	8.35	4.01–15.07	24/41
Creatinine (μmol/l)	121.67 ± 18.53	120.2	78.3–157.7	24/41
Urea (mmol/l)	5.89 ± 1.85	5.3	2.93–9.92	24/41
Bilirubin (μmol/l)	17.7 ± 7.9	15.95	6.6–37.7	24/40
ALP (μkat/l)	3.53 ± 0.92	3.37	1.89–5.59	24/41
ALT (μkat/l)	0.21 ± 0.07	0.2	0.09–0.35	15/22
AST (μkat/l)	5.69 ± 0.98	5.76	3.75–8.43	23/39
GMT (μkat/l)	0.27 ± 0.08	0.26	0.15–0.51	24/41
CK (μkat/l)	4.97 ± 1.99	4.57	2.22–9.76	21/37
LDH (μkat/l)	14.79 ± 2.75	14.54	10.64–20.26	22/38
Cholesterol (mmol/l)	1.83 ± 0.32	1.77	1.27–2.57	24/40
Na (mmol/l)	143.97 ± 2.35	145	136–145	23/37
K (mmol/l)	4.35 ± 0.44	4.4	3.5–5.1	24/41
Ca (mmol/l)	2.64 ± 0.21	2.65	2.24–3.1	24/41
P (mmol/l)	1.16 ± 0.38	1.05	0.47–1.96	23/40
Cl (mmol/l)	100.01 ± 6.74	99.8	85.6–113.9	22/35
Zn (μmol/l)	9.44 ± 2.34	9.18	5.36–16.32	23/39
Cu (μmol/l)	16.41 ± 4.52	15.87	8.7–31.88	23/37
Mg (mmol/l)	0.64 ± 0.08	0.65	0.47–0.82	22/37
Vitamin A (μmol/l)	1.29 ± 0.38	1.31	0.59–2.39	24/39
Vitamin E (μmol/l)	8.35 ± 3.65	7.38	3.5–17.88	24/39

Minimum and maximum values, *n* = number of horses/number of samples

of sodium ($P < 0.01$) and creatinine ($P < 0.05$) were also higher.

Concentrations of total protein, albumin, globulin and their ratio in the plasma of Przewalski horses do not differ from most references. Ranges of all parameters are slightly wider than in the domestic horses. Total protein levels were only lower when compared with the data of Kuttner and Wiesner (1987). Likewise, ranges of ions in the serum correspond with reference limits for the domestic horses. Apart from calcium and magnesium, median values are slightly higher.

The most profound difference concerned the concentration of glucose. It was considerably higher than in a previous study on the Przewalski horses (Kuttner and Wiesner 1987) and nearly twice higher when compared with the median value for the domestic horses (Lumsden et al. 1980). The higher glycaemia may have resulted from the stress of separation from the herd as well as immobilisation (Kuttner and Wiesner 1987).

Enzyme activities in the Przewalski horses (in the present study and published data, Table 8) are higher than in domestic horses, but not considerably different from the species-specific data. It is, therefore, clear that reference ranges from domestic horses are not suitable for the evaluation of findings in the Przewalski horses. The situation of total bilirubin is similar, but its concentration is higher in domestic horses (Kuttner and Wiesner 1987; Lumsden et al. 1987; Baronetzky-Mercier 1992).

In contrast, concentrations of urea and creatinine are within reference ranges for domestic horses. The median values of these two parameters are higher in the present study.

The concentration of cholesterol that we found was lower than reported by other authors (Lumsden et al. 1987; Baronetzky-Mercier 1992).

Table 6. Comparison of biochemical parameters of mares and stallions

	♀			♂			Statistical evaluation	
	$\bar{x}$ ± SD	min–max	*n*	$\bar{x}$ ± SD	min–max	*n*	*F*-test	*t*-test
Total protein (g/l)	63.8 ± 8	48.9–81	17/27	64.7 ± 5.57	57.5–77.5	7/14	0.172	0.342
Albumin (g/l)	32.3 ± 3.79	24.5–38.5	17/27	31.74 ± 2.9	27.4–35.8	7/14	0.315	0.347
Globulin (g/l)	31.9 ± 6.13	20.5–43.7	15/24	32.04 ± 4.64	24.8–39	6/12	0.337	0.465
A/G	1.04 ± 0.2	0.57–1.39	15/24	1 ± 0.25	0.57–1.42	7/13	0.419	0.268
Glucose (mmol/l)	9.15 ± 2.93	4.45–15.1	17/24	7.76 ± 2.22	4.01–12.78	7/14	0.296	0.065
Creatinine (µmol/l)	125 ± 17.4	91.4–158	17/27	115.2 ± 19.6	78.3–148.8	7/14	0.578	0.053
Urea (mmol/l)	6.06 ± 2.08	2.93–9.92	17/27	5.55 ± 1.33	3.57–7.81	7/14	0.093	0.207
Bilirubin (µmol/l)	16.2 ± 6.86	6.6–28.7	17/26	20.57 ± 9.12	8.5–37.7	7/14	0.214	0.046*
ALP (µkat/l)	3.61 ± 0.98	1.89–5.59	17/27	3.38 ± 0.83	2.05–4.81	7/14	0.541	0.235
ALT (µkat/l)	0.19 ± 0.07	0.09–0.35	9/12	0.23 ± 0.06	0.11–0.33	7/10	0.674	0.121
AST (µkat/l)	5.69 ± 0.89	4.04–7.12	16/25	5.68 ± 1.61	3.75–8.43	7/14	0.259	0.487
GMT (µkat/l)	0.24 ± 0.05	0.15–0.36	17/27	0.34 ± 0.08	0.21–0.51	7/14	0.127	2.14×10^{-5}**
CK (µkat/l)	5.3 ± 1.88	2.89–9.76	15/23	4.42 ± 2.11	2.22–9.41	7/14	0.613	0.099
LDH (µkat/l)	15.9 ± 2.67	10.6–20.3	16/25	12.69 ± 1.38	10.68–15.47	7/13	0.020*	1.17×10^{-5}**
Cholesterol (mmol/l)	1.97 ± 0.29	1.44–2.57	17/27	1.55 ± 0.17	1.27–1.87	7/13	0.051	7.19×10^{-7}**
Na (mmol/l)	145 ± 1.05	142–147	16/23	142.7 ± 3.27	136–145	7/14	5.59×10^{-6}**	0.020*
K (mmol/l)	4.35 ± 0.46	3.5–5.1	17/27	4.35 ± 0.41	3.66–5.04	7/14	0.691	0.482
Ca (mmol/l)	2.64 ± 0.23	2.32–3.1	17/27	2.63 ± 0.18	2.24–2.93	7/14	0.282	0.442
P (mmol/l)	1.15 ± 0.37	0.47–1.91	17/27	1.19 ± 0.40	0.75–1.96	6/13	0.756	0.400
Cl (mmol/l)	99.1 ± 6.75	85.6–109	15/22	101.6 ± 6.68	89.1–113.9	7/13	0.991	0.142
Zn (µmol/l)	9.23 ± 2.08	5.36–13.8	16/26	9.86 ± 2.84	6.12–16.32	7/13	0.185	0.220
Cu (µmol/l)	16.2 ± 2.13	12.6–18.8	16/24	16.86 ± 7.22	8.7–31.88	7/13	1.02×10^{-6}**	0.370
Mg (mmol/l)	0.62 ± 0.09	0.47–0.82	15/24	0.67 ± 0.05	0.58–0.8	7/13	0.063	0.045*
Vitamin A (µmol/l)	1.37 ± 0.4	0.72–2.39	17/26	1.15 ± 0.30	0.59–1.62	7/13	0.286	0.044*
Vitamin E (µmol/l)	8.8 ± 4.08	3.5–17.9	17/26	7.45 ± 2.49	3.88–11.63	7/13	0.076	0.140

Minimum and maximum values, *n* = number of horses/number of samples, *$P < 0.05$, **$P < 0.01$

Table 7. Comparison of biochemical parameters of one- to five-year-old horses and horses older than five year

	One- to five-year-old horses			Horses older than five years			Statistical evaluation	
	$\bar{x}$ ± SD	min–max	*n*	$\bar{x}$ ± SD	min–max	*n*	*F*-test	*t*-test
Total protein (g/l)	64 ± 5.59	55.2–71.2	9/11	64.1 ± 7.79	48.9–81	16/30	0.267	0.473
Albumin (g/l)	33.38 ± 2.34	29.4–36.7	9/11	31.55 ± 3.73	24.5–38.5	16/30	0.120	0.069
Globulin (g/l)	31.15 ± 4.21	24.8–37	7/8	32.74 ± 6.71	20.5–49.4	16/29	0.204	0.265
A/G	1.12 ± 0.18	0.9–1.42	7/8	1 ± 0.22	0.57–1.4	16/29	0.603	0.102
Glucose (mmol/l)	8.85 ± 2.21	4.67–12.78	9/11	8.61 ± 2.98	4.01–15.07	16/30	0.320	0.403
Creatinine (µmol/l)	132.3 ± 15.36	107.3–157.7	9/11	117.8 ± 18.27	78.3–156.7	16/30	0.579	0.012*
Urea (mmol/l)	6.3 ± 2.16	4.22–9.92	9/11	5.74 ± 1.74	2.93–9.65	16/30	0.350	0.197
Bilirubin (µmol/l)	18.72 ± 6.68	8.4–28.7	9/11	18.09 ± 9.25	6.6–40.3	16/30	0.278	0.419
ALP (µkat/l)	3.94 ± 0.93	2.87–5.59	9/11	3.38 ± 0.89	1.89–5.16	16/30	0.809	0.041*
ALT (µkat/l)	0.47 ± 0.51	0.09–1.3	4/5	0.22 ± 0.07	0.1–0.35	13/19	1.42×10^{-9}**	0.164
AST (µkat/l)	5.86 ± 1.74	2.47–9.2	9/11	5.54 ± 0.85	3.75–7.26	16/29	0.003**	0.281
GMT (µkat/l)	0.25 ± 0.04	0.17–0.31	8/10	0.27 ± 0.07	0.15–0.41	16/30	0.027	0.126
CK (µkat/l)	4.47 ± 1.24	3.23–6.65	7/8	5.11 ± 2.15	2.22–9.76	15/29	0.139	0.214
LDH (µkat/l)	14.51 ± 3.08	10.68–18.54	8/9	15.06 ± 2.84	10.64–20.39	16/30	0.693	0.309
Cholesterol (mmol/l)	1.89 ± 0.32	1.44–2.57	9/11	1.82 ± 0.32	1.27–2.41	16/30	0.969	0.279
Na (mmol/l)	145.3 ± 0.71	145–147	8/8	143.4 ± 2.71	136–145	16/30	0.001**	0.001**
K (mmol/l)	4.24 ± 0.51	3.61–5.1	9/11	4.33 ± 0.41	3.5–5.1	16/30	0.339	0.264
Ca (mmol/l)	2.74 ± 0.21	2.44–2.98	9/11	2.60 ± 0.21	2.24–3.1	16/30	0.945	0.040*
P (mmol/l)	1.41 ± 0.37	0.7–1.96	9/11	1.07 ± 0.34	0.47–1.92	15/29	0.695	0.004**
Cl (mmol/l)	102.5 ± 5.90	92.6–111.1	8/10	99.01 ± 6.91	85.6–113.9	14/25	0.642	0.084
Zn (µmol/l)	10.08 ± 2.23	7.14–13.77	8/10	8.97 ± 1.99	5.36–12.75	15/28	0.606	0.075
Cu (µmol/l)	16.88 ± 1.41	14.13–18.84	8/10	17.18 ± 6.18	8.7–32.03	16/29	7.42×10^{-5}	0.405
Mg (mmol/l)	0.63 ± 0.07	0.52–0.76	7/8	0.64 ± 0.09	0.47–0.82	16/29	0.619	0.348
Vitamin A (µmol/l)	1.19 ± 0.28	0.72–1.62	9/11	1.33 ± 0.41	0.59–2.39	16/28	0.192	0.151
Vitamin E (µmol/l)	7.43 ± 3.20	3.5–12	9/11	8.71 ± 3.81	3.75–17.88	16/28	0.581	0.166

Minimum and maximum values, *n* = number of horses/number of samples, *$P < 0.05$, **$P < 0.01$

Table 8. Comparison of biochemical parameters based on all specimens examined (without consideration of age and gender) with published data on the Przewalski horses

	E.p. ZOO Prague (n = 22–41)	E.p. Kuttner (n = 20)	E.p. ISIS in Baronetzky-Mercier (n = 39–78)	E.p. Baronetzky-Mercier (n = 4)	E.p. Hawkey in Baronetzky-Mercier (n = 4)
Total protein (g/l)	64.1 ± 7.2	69 ± 1	64 ± 6	71 (64–81)	64 ± 5
Albumin (g/l)	32 ± 3.5	N	33 ± 3	36 (33–37)	31 ± 2
Globulin (g/l)	31.9 ± 5.6	N	N	N	33 ± 5
A/G	1 ± 0.2	N	N	N	1 ± 0.2
Glucose (mmol/l)	8.68 ± 2.77	6.22 ± 1.17	8.27 ± 3.4	N	4.55 ± 0.52
Creatinine (µmol/l)	121.67 ± 18.53	99.01 ± 25.64	106.01 ± 17.68	141.44–265.2	115 ± 18
Urea (mmol/l)	5.89 ± 1.85	4.13 ± 2.75	5.92 ± 1.35	4.9–11.32	N
Bilirubin (µmol/l)	17.7 ± 7.9	16.76 ± 6.16	23.94 ± 3.42	N	32.5 ± 8.6
ALP (µkat/l)	3.53 ± 0.92	5.92 ± 2.22	4.15 ± 2.88	7.02–9.94	0.76 ± 0.18
ALT (µkat/l)	0.21 ± 0.07	0.13 ± 0.05	0.22 ± 0.18	0.18–0.67	0.072 ± 0.06
AST (µkat/l)	5.69 ± 0.98	3.47 ± 0.82	5.55 ± 2.08	2.83–4.67	3.62 ± 2.33
GMT (µkat/l)	0.27 ± 0.08	0.28 ± 0.1	N	0.33–0.63	N
CK (µkat/l)	4.97 ± 1.99	2.37 ± 0.9	N	N	0.65 ± 0.67
LDH (µkat/l)	14.79 ± 2.75	9.65 ± 2.35	7.82 ± 2.48	2.57–16.64	2.33 ± 0.56
Cholesterol (mmol/l)	1.83 ± 0.32	N	2.44 ± 0.43	2.28 (2.18–2.36)	2.25 ± 0.34
Na (mmol/l)	143.97 ± 2.35	138 ± 4	136 ± 2.9	140 (136–143)	140 ± 1.5
K (mmol/l)	4.35 ± 0.44	4.7 ± 0.5	4.6 ± 0.6	N	3.6 ± 0.4
Ca (mmol/l)	2.64 ± 0.21	3 ± 0.1	2.8 ± 0.23	2.38 (1.8–3.05)	2.98 ± 0.13
P (mmol/l)	1.16 ± 0.38	1.52 ± 0.42	1.55 ± 0.55	N	1.03 ± 0.16
Cl (mmol/l)	100.01 ± 6.74	94 ± 4	97 ± 3.4	N	99 ± 1
Zn (µmol/l)	9.44 ± 2.34	N	N	N	N
Cu (µmol/l)	16.41 ± 4.52	N	N	N	N
Mg (mmol/l)	0.64 ± 0.08	N	N	N	0.82 ± 0.04
Vitamin A (µmol/l)	1.29 ± 0.38	N	N	N	N
Vitamin E (µmol/l)	8.35 ± 3.65	N	N	N	N

E.p. = *Equus przewalski*, N = not available

Published data on the concentration of vitamins A and E in the plasma of horses vary. The plasmatic levels of these vitamins are to a great degree influenced by the feeds, pasture, season and feed additives. Compared with our data, Dierenfeld et al. (1997) reported a higher level of vitamin E in Przewalski horses free at pasture (8.35 ± 3.65 versus 14.61 ± 3.09 µmol/l). These authors blood-sampled the horses during June, i.e., the period of the highest quality of pasture, while our data are based on collections during the whole year. It is conceivable that this difference may be responsible for the difference. Indeed, mares at pasture show the highest levels of fat soluble vitamins during summer months (Maenpaa et al. 1988). Blakley and Bell (1994) reported lower concentrations of vitamin A (0.70 µmol/l) and E (7.65 µmol/l) in domestic horses.

There are no reports on the plasma concentrations of copper and zinc in the Przewalski horses. Concentrations of plasma microelements vary in domestic horses and are influenced by both the feeding ration and physiological states such as pregnancy and lactation. Table 9 shows that the concentrations of copper and zinc in the Przewalski horses are approximately in the centre of ranges measured in domestic horses by various authors (Grace et al. 1999; Yur et al. 2008; Aytekin et al. 2010).

Table 9. Comparison of concentrations of microelements based on all specimens examined (without consideration of age and gender) with published data concerning both the Przewalski and domestic horses

	E.p. ZOO Prague (n = 39)	E.c. Grace[1] (n = 21)	E.c. Aytekin[2] (n = 15)	E.c. Yur[3] (n = 25)	E.c. Yur[4] (n = 25)
Zn (μmol/l)	9.44 ± 2.34	7.50 ± 0.35	14.08 ± 3.57	9.49 ± 0.31	7.96 ± 0.46
Cu (μmol/l)	16.41 ± 4.52	17.43 ± 6.44	18.41 ± 1.03	11.62 ± 0.47	23.71 ± 3.61

E.p. = *Equus przewalski*, E.c. = *Equus caballus*
[1]mares on days 135 to 150 of lactation, pasture+supplements
[2]hay, grain, mineral lick
[3]without vitamin E + Se supplementation
[4]single vitamin E + Se supplementation

CONCLUSION

The data reported herein clearly document certain differences in haematological and biochemical parameters between Przewalski and domestic horses. Available reference ranges for the domestic horses are, therefore, not suitable for the evaluation of findings in Przewalski horses. The use of data reported for the Przewalski horses by different authors can also be misleading. These data have frequently been obtained from a small sample size of "clinically healthy" specimens. It is very difficult to evaluate the state of health of animals kept in zoos and parks. Zoo animals are often examined under general anaesthesia and it is not possible to exclude stress subsequent changes in physiological parameters. It is even impossible to exclude subclinical diseases. Animals may be kept under different conditions and climatic zones; likewise, the feeding rations vary. All these factors can influence the ranges of measured parameters. It is, therefore, advisable to obtain reference ranges from a population kept under specific set conditions.

Other authors evaluated less parameters and used a smaller sample size in their studies on Przewalski horses. Therefore, with respect to the sample size and the number of animals analysed we believe that our study is unique. Likewise, ours is the first study to evaluate gender and age groups in Przewalski horses. It is, therefore, difficult to compare our results with other studies.

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Corresponding Author:

Jaroslava Tomenendalova, University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Palackeho1/3, 612 42 Brno, Czech Republic
E-mail: tomenendalovaj@vfu.cz